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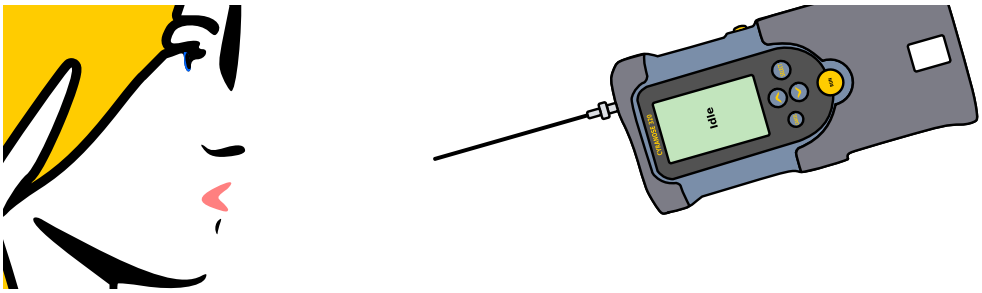
NONINVASIVE DISCRIMINATION OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND α_1-ANTITRYPSIN DEFICIENCY USING AN ELECTRONIC NOSE



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"Fifty – fifty, gut – böse, ja – nein, an – aus, Kopf oder Zahl,
Mann oder Maus. Ich spiel' mal Loser, mal Gewinner.
Mal spiel' ich den Normalen und dann den Spinner, doch
ich selbst bleib' ich immer."

Blumentopf 1999

Abstract

Objectives

The aim of this thesis was to assess, if human exhaled breath of patients with chronic obstructive pulmonary disease (COPD) and of patients with α_1-antitrypsin deficiency (AATD), that resembles COPD, can be used for simplified, noninvasive, and cost-efficient diagnostic purposes using electronic nose technology.

Methods

Exhaled breath condensate and pure exhaled breath of patients with COPD (n = 10), with AATD (n = 23), and healthy controls (n = 10) were sampled and analyzed by measuring volatile organic compounds (VOCs) via the Cyranose 320 electronic nose and the BioScout ion mobility spectrometry (IMS) device. Furthermore, the effect of the α_1-antitrypsin augmentation therapy on the exhaled breath (EB) profile of eleven AATD patients was examined. In addition, EB profiles were analyzed by linear discriminant analysis (LDA), Mahalanobis distance (MD), and cross-validation value (CVV) of a k-fold cross-validation.

Results

VOC profiles from AATD patients were significantly different from COPD patients in exhaled breath condensate (LDA: $p < 0.0001$, sensitivity of 1.00, specificity of 1.00, CVV 82.0 %, MD 2.37) and in pure exhaled breath (LDA: $p < 0.0001$, sensitivity of 1.00, specificity of 1.00, CVV 58.3 %, MD 2.27). Moreover, VOC profiles of AATD patients before and after augmentation therapy differed significantly (LDA: $p = 0.001$, sensitivity of 1.00, specificity of 1.00, CVV 53.3 %, MD 1.79). The discrimination via the BioScout IMS device supported the results.

Conclusion

It was demonstrated, that an electronic nose and IMS device in combination with data analysis described in this work can discriminate exhaled breath profiles of COPD patients from patients with AATD. Additionally, the augmentation therapy changed the exhaled breath profile of AATD patients. These results suggest, that electronic noses could be used to improve the noninvasive diagnosis of AATD and COPD.

Zusammenfassung

Zielsetzung

Das Ziel dieser Arbeit war es, die Ausatemluft von Patienten mit chronischer obstruktiver Lungenerkrankung (engl. chronic obstructive pulmonary disease, COPD) mit der von Patienten mit α_1-Antitrypsinmangel (engl. α_1-antitrypsin deficiency, AATD), welcher der COPD sehr ähnelt, zu untersuchen. Hierfür wurde die Ausatemluft mit einer elektronischen Nase gemessen und überprüft, ob hieraus ein vereinfachter, nicht-invasiver und kostengünstiger diagnostischer Nutzen gezogen werden kann.

Methoden

Exhaliertes Atemwegskondensat und direkte Ausatemluft wurden von Patienten mit COPD ($n = 10$), mit AATD ($n = 23$) und von gesunden Kontrollen ($n = 10$) mittels einer elektronischen Nase (Cyranose 320) und Ionenmobilitätsspektrometrie (IMS) (Bioscout) untersucht. Der Effekt der AAT-Substitutionstherapie auf das Ausatemluft-Profil von elf AATD-Patienten wurde ebenfalls analysiert. Die Profile wurden per linearer Diskriminanzanalyse (LDA), Mahalanobis-Distanz (MD) und Kreuzvalidierungswert (engl. cross-validation value, CVV) einer k-fachen Kreuzvalidierung untersucht.

Ergebnisse

Die Profile aus Kondensat-Proben von AATD-Patienten unterschieden sich von denen von COPD-Patienten (LDA: $p < 0,0001$, Sensitivität von 1,00, Spezifität von 1,00, CVV 82,0 %, MD 2,37). Die Profile aus direkter Ausatemluft unterschieden sich ebenfalls (LDA: $p < 0,0001$, Sensitivität von 1,00, Spezifität von 1,00, CVV 58,3 %, MD 2,27). Die gemessenen Ausatemluft-Muster bei AATD-Patienten vor und nach der Substitutionstherapie veränderten sich statistisch signifikant (LDA: $p = 0,001$, Sensitivität von 1,00, Spezifität von 1,00, CVV 53,3 %, MD 1,79). Die Resultate der IMS stützten diese Ergebnisse.

Schlussfolgerung

Es konnte gezeigt werden, dass die elektronische Nase und IMS in Kombination mit den hier gezeigten Methoden zur Datenanalyse Ausatemluft von COPD-Patienten von der Ausatemluft von AATD-Patienten unterscheiden kann. Die Substitutionstherapie veränderte ebenfalls das Ausatemluft-Profil von AATD-Patienten. Die elektronische Nase mag somit bei einer nicht-invasiven Diagose von AATD hilfreich sein.

Contents

1 Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous and life-threatening disease with an estimated global prevalence of 4.77 % [150]. The diagnosis is complex, time-consuming, and cost-intensive. A closely related and genetic disease, α_1-antitrypsin deficiency (AATD), shares similar clinical symptoms [8, 20, 100, 140]. The diagnosis of AATD is complex as well. For an appropriate therapy it is crucial to distinguish between these two diseases, which is currently very difficult and elaborate.

As it is known for different forms of cancer to be recognized via odors [99, 120], it is the aim of the thesis at hand to test the hypothesis, if it is possible to distinguish COPD from AATD noninvasively and cost-efficiently via odors in exhaled breath (EB) [59]. These differentiating odors can be measured using so-called electronic noses (ENs).

Hence, the following main questions regarding the usage of an EN will be answered:

- Can exhaled breath condensate be used to discriminate between patients with AATD, with COPD, and healthy controls?
- Can total exhaled breath also be used to distinguish between patients with AATD, with COPD, and healthy controls?
- Does augmentation therapy has a significant impact on exhaled breath of AATD patients?

1.1 Chronic obstructive pulmonary disease

COPD is in most cases a preventable disease. It is is characterized by a chronic irritation of the airways featuring persistent and progressive airflow limitation, that is associated with an enhanced chronic inflammatory response in the airways and the lung to noxious agents [51]. COPD comprises chronic obstructive bronchitis and lung emphysema and is featured by a largely irreversible and progressive obstruction of the lower airways [51].

The prevalence in Germany was estimated at 7.8 % in 2007 and is predicted to increase to 11.5 % by 2050 [14]. Additional epidemiological parameters include region, sex, age, and death toll and have been discussed in detail elsewhere [41, 95, 98, 102].

1.1.1 Etiology

The primary risk factor of COPD is tobacco smoking [51]. Other toxic agents [23, 73, 75, 128, 129, 147], rare genetic factors, like α_1-antitrypsin deficiency (see section 1.2) [68, 124], severe infections, and exacerbations cause or contribute to COPD [51, 101].

1.1.2 Diagnostics

COPD should be considered in patients with chronic cough or sputum production, dyspnea and/or a background of exposure to noxious agents [51]. The current clinical diagnosis usually includes pulmonary function testing via spirometry and body plethysmography to assess airflow obstruction and emphysema [51]. These procedures are complex, time-consuming, and require the patient to inhale pharmacological agents [51].

Other parameters, such as exacerbation rate and questionnaires, help to assess the severity of the disease [19, 47, 51, 71, 72, 111].

COPD can be divided into four severity grades of airflow limitation (I to IV) [51] or in four risk and symptom groups. These groups are based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) severity grade, exacerbation rate, and one of two questionnaires [51].

1.1.3 Symptoms and pathophysiology

Typically, COPD is accompanied by cough, sputum production, and progressive dyspnea as a result of inflammatory processes in the lung [51]. These pulmonary symptoms usually occur in patients between 40 years and 60 years of age [51]. Pathological changes affect the airways, lung parenchyma, and pulmonary vasculature, featuring airflow limitation, air trapping, and pulmonary hypertension [61].

Oxidative stress from exogenous reactive oxygen species (ROS) [123] advance inflammatory processes [156]. In addition, the activation of inflammatory cells produces endogenous ROS, which on its part contribute to the progression of COPD [125]. Repeated exposure to ROS causes a chronic inflammatory response, that leads to structural changes due to permanent damage and recovery [10, 51] resulting in an impaired gas exchange [148] and a disturbed homeostasis of proteases and protease inhibitors in the lung, which causes centrilobular emphysema [26, page 231]. In rare cases this imbalance can be linked to α_1-antitrypsin deficiency (see section 1.2).

1.2 α_1-antitrypsin deficiency

α_1-antitrypsin (AAT) is a systemic serine protease inhibitor (serpin). It is mainly expressed in the liver [62, 82], but it is also found in other cells [24, 25, 115]. AAT acts as an acute-phase glycoprotein, primarily as an inhibitor of the proteolytic enzyme neutrophil elastase [28, 68]. This enzyme is secreted by neutrophil granulocytes

[145] and it cleaves neighboring tissue in order to let the secreting cells reach sites of inflammation [12, 13].

However, if not regulated properly, neutrophil elastase may cause damage to the host tissue [74]. The equilibrium between neutrophil elastase (protease) and AAT (protease inhibitor) allows the elastase to serve its key function against pathogens, but leaves host tissue undamaged to the greatest possible extent. This is crucial in lung tissue, as it is steadily exposed to environmental proinflammatory stimuli.

α_1-antitrypsin deficiency (AATD) is a rare co-dominantly inherited genetic disorder. This disease is characterized by mutations of the AAT gene. Within more than one hundred characterized mutations of AAT [7] the two major pathological forms are called S- and Z-type, which are misfolded and less functional [70, 114]. The latter represents a very severe and the clinically most relevant kind for this disorder [31]. The physiological form of AAT is called M-type.

The estimated global prevalence for AATD is 0.08 %. Germany has a prevalence of 0.10 % (data taken from [15]). Detailed data from different regions, on sex, and rates of carriers of a defective gene are given in [15, 29, 40].

1.2.1 Etiology

Besides genetic factors, the same contributing factors for COPD (see section 1.1.1) apply for AATD [113]. Pulmonary symptoms usually occur about ten years earlier [69, 84, 143]. Exacerbations go along with increased inflammatory activity in the airways [60], hence, leading to a raised secretion of neutrophil elastase damaging host tissue.

1.2.2 Diagnostics

The current diagnosis of α_1-antitrypsin deficiency is very complex and cost-intensive. It can take up to several years until the disorder gets diagnosed properly [20, 140, 142], due to rare incidence and unspecific symptoms [8, 20, 55, 100, 140].

For this reason, the GOLD guidelines and the World Health Organization (WHO) recommend, that every COPD patient should be screened for AATD [51], especially in high prevalence regions, if near relatives are known to suffer from AATD, or when pulmonary symptoms occur already before midlife [124, 141]. It is assumed, that about 1 % to 2 % of all COPD patients are suffering from AATD [68, 139].

Along with lung function assessment, a diagnosis usually involves serum analysis via nephelometry for the initial determination of the AAT level, isoelectric focusing (IEF)

for phenotyping, polymerase chain reaction (PCR) for genotyping, and in rare cases genome sequencing [8].

1.2.3 Symptoms and pathophysiology

The pulmonary symptoms of AATD are commonly similar to those of COPD and also asthma [100]. These symptoms may already occur in patients at the age of 30 years to 40 years [68]. The mutated gene usually causes abnormal types of the AAT protein, which is typically accompanied by low serum levels of the protein [8]: levels of Z-type diseased individuals reach only about 10 % of healthy ones. Severe mutations regularly cause the misfolded AAT proteins to polymerize and to thereby lose their inhibitory ability [39, 68, 86, 87, 88]. This may lead to a specific imbalance of proteases (neutrophil elastase) and (functional) protease inhibitors. It follows an improperly controlled proteolytical activity, especially in the lung, which then typically causes COPD with panlobular emphysema [48, 109]. In this regard, AATD can be considered a genetic variant of COPD [51].

Since AAT is mainly expressed in liver cells, AATD may cause liver disease [21, 28, 119]. In addition, oxidized and polymers of misfolded AAT proteins seem to have proinflammatory effects [1, 108, 116].

The key differences between the clinically similar diseases—COPD and AATD—are summarized in table 1.2.1.

Table 1.2.1: Comparison of COPD and AATD

	COPD	AATD
Estimated global prevalence	4.77 %	0.08 %
Main cause	noxious gases and particles	dysfunctional AAT protein
	mostly preventable	genetically inherited
Age at onset of pulmonary symptoms in years	40 to 60	30 to 40
Pathophysiology	ROS-induced chronic inflammation	protease–antiprotease imbalance
Emphysema	centrilobular	panlobular

COPD: chronic obstructive pulmonary disease, AATD: α_1-antitrypsin deficiency, AAT: α_1-antitrypsin, ROS: reactive oxygen species.

1.2.4 Treatment

Apart from the standard therapy and management for COPD, a symptomatic and more specific treatment consists of the administration of the lacking AAT by infusion. However, the results on therapeutic efficacy across different publications diverge [3, 33, 144, 152]. Generally, there is strong evidence of biochemical effects and safety of the AAT infusion therapy [32].

1.3 Odors as disease markers

Odors are caused by at least one volatile chemical substance, that can be recognized by the olfactory system [94] and serve different purposes [97, 112, 149, 132, page 1].

Odors from the body arise as a mixture of odorous chemical compounds, secreted from numerous cells in various metabolic pathways and can be affected by diet or diseases [16, 58, 89, 110, 131, 133, 134, 146, 151].

1.3.1 Disorders

Already in the 5th century before the Common Era physicians noticed, that specific diseases can have an impact on body odors [45, pages 35, 63, 66f., 72, and 110]. The "maple syrup urine disease", for instance, is named after the characteristic smell of the urine [103, 122]. Other disorders also have specific odors [38, 49, 91, 104]. Furthermore, the exhaled odors of individuals with cancer differ from that of healthy controls [99, 120, 138].

1.3.2 Volatile organic compounds

Volatile organic compounds (VOCs) are organic (carbonic) substances, that easily vaporize, such as ethanol. Although there is no international standardized definition, VOCs will be defined for the remainder of this thesis as organic compounds, that evaporate at room temperature or higher.

There are VOCs in human exhaled breath [52, 99, 120], that can be detected and measured to assess human EB via electronic noses [59] (see section 1.4) or ion mobility spectrometry (IMS) [79] (see section 3.2).

1.3.3 Exhaled breath condensate

Human exhaled breath (EB) (also pure exhaled breath (PEB)) consists of nitrogen ($\approx 74\,\%$), oxygen ($\approx 15\,\%$), carbon dioxide ($\approx 4\,\%$) [136, page 107], water vapor

(≈ 6 %), and organic components [117]. EB also contains certain mixtures of VOCs, that are influenced by specific diseases or conditions, especially of the lung [92, 154].

Exhaled breath condensate (EBC) is the liquid, that results from cooling exhaled breath (EB). Thereby, exhaled water vapor including all of its dissolved compounds condensates. EBC consists of more than 99 % water and different soluble compounds and provides multiple markers [6, 22, 37, 50, 63, 80, 81, 96, 106, 107, 159]. Collecting EBC is well established and mostly standardized [63].

Analyzing EBC provides a noninvasive, easily repeatable, simple, and cost-effective option to assess the lung and the airway lining fluid. Two major advantages are, that EBC can be easily obtained and stored for later analyses [63]. Due to its harmless nature, collecting EBC can be performed without impact on the body. This makes the method suitable for monitoring lung conditions via follow-up examinations, which may include assessment with an electronic nose.

1.4 Electronic noses

Electronic noses (ENs) are odor detectors, *i.e.*, devices to artificially mimic the mammalian olfactory sense. Usually, ENs are able to detect VOCs (see section 1.3.2) via an array of cross-reactive conducting polymer sensors [85] (see section 3) resulting in "smellprints".

These smellprints can be used for computer-aided analysis in regard to comparison and discrimination of profiles. Those analyses allow the generation of prediction models to classify new and unknown samples.

It is important to note, that ENs are usually not able to identify single components out of a complex VOC mixture as they use cross-reactive sensors.

There are no generally accepted recommendations or guidelines for sample taking. Nevertheless, a commercially successful device, namely the Cyranose 320 (C-320) EN [67], has been used by other research groups effectively and it turned out to be suitable and cost-efficient for various applications [34, 35, 36, 43, 59, 83, 135]. Therefore, this device was used for this work, too.

Several techniques and standard operation procedures (SOPs) were established within the scope of this work, such as collecting and measuring EBC and standardizing the assessment of human EB via a specific EN.

1.5 Ion mobility spectrometry

Besides “classical” ENs, devices using ion mobility spectrometry (IMS) use a fundamentally different concept to analyze VOCs. By means of ionization, the IMS is able to separate molecules on the basis of their physical properties, like mass, size, shape, and mobility in the gas phase (drift time) [11, 27].

As several analytes can be characterized by the same drift time, a preseparation, such as gas chromatography (GC) using a multi-capillary column (MCC), for instance, can improve the sensitivity in terms of qualitative features [11]. Different substances need different times to pass the MCC (retention time). Hence, this combination (gas chromatography (GC)/MCC + IMS) provides the characterization of substances by their retention and drift times. An additional, parallel analysis of VOC samples by mass spectrometry (MS) associated with gas chromatography (GC-MS) even allows the identification of single substances in a VOC mixture [11].

The BioScout IMS device [4] is a commercially available instrument. This device is able to detect discriminating substances for COPD (cyclohexanone) [155], sarcoidosis [18], and lung cancer [153] in human EB.

One major advantage of the BioScout IMS device is the capability to decompose complex VOC mixtures down to single molecules. In addition, sampling of breath is more advanced, as it allows to analyze specific fractions of exhaled breath, *e.g.*, breath from the lower airways only.

2 Results

2.1 Exhaled breath condensate

A total of 30 volunteers participated in the EBC experiment, 10 in each group, in particular, COPD, AATD, and healthy controls (HCs). On average, the HCs (mean age 30 years) were younger than patients with COPD (mean age 55 years) or AATD (mean age 66 years). Regarding the $\%FEV_1$ (defined as the ratio of the actual forced expiratory volume in one second (FEV_1) and the predicted FEV_1), on average the HCs (105 %) had higher values compared to patients with COPD (51 %) or AATD (48 %). The proportion of females was higher in HCs (60 %) than in both other groups (each with 20 %). Only two COPD patients were smoking at the time of sampling including one pipe smoker. All other AATD and COPD patients and HCs had quit smoking or were never-smokers. The patient characteristics are summarized in table 2.1.1. The difference in the $\%FEV_1$ of AATD and COPD patients reached no statistical significance ($p = 0.08$)[1]. A logistic regression analysis[2] showed, that the $\%FEV_1$ had no statistically significant impact ($p = 0.46$) on the differentiation of COPD and AATD patients.

Table 2.1.1: Exhaled breath condensate participants

	AATD	COPD	HC
Mean age [a]	55	66	30
(standard deviation)	(8)	(6)	(7)
Number ♀ / ♂	2 / 8	2 / 8	6 / 4
Mean $\%FEV_1$ [%]	47.9	51.1	105.2
(standard deviation)	(16.6)	(10.9)	(9.9)
Smoker current / ex	0 / 9	2* / 8	0 / 2

$\%FEV_1$ is defined as the ratio of the actual FEV_1 and the predicted FEV_1. AATD: α_1-antitrypsin deficiency, COPD: chronic obstructive pulmonary disease, HC: healthy control, FEV_1: forced expiratory volume in one second, * including 1 pipe smoker.
Data taken from [59].

[1] Statistical significance is considered with a p-value less than or equal to 0.05.
[2] A logistic regression is a statistical analysis method for categorical variables.

Table 2.1.2: Exhaled breath condensate results

Group comparison	Linear discriminant analysis p-value	CVV	Sensitivity	Specificity	MD
AATD vs COPD	< 0.0001	82.0 %	1.00	1.00	2.37
AATD vs HC	< 0.0001	59.5 %	1.00	1.00	2.12
COPD vs HC	< 0.0001	80.5 %	1.00	1.00	2.19

Pairwise comparisons and statistical analyses of the linear discriminant analysis data.
CVV: cross-validation value, MD: Mahalanobis distance, AATD: α_1-antitrypsin deficiency, COPD: chronic obstructive pulmonary disease, HC: healthy control. Data taken from [59].

The linear discriminant analysis (LDA)[3] was able to separate the three groups (see publication figure 2 (page 29/1261) in reference to the data obtained with the C-320 EN. Each of the groups, namely, AATD, COPD, and HCs, formed a separate cluster and was clearly distinguishable. The corresponding Mahalanobis distances (MDs)[4] were ranging from 2.12 to 2.37: The MD between AATD and COPD was 2.37, the MD between AATD and HC was 2.12, while the MD between COPD and HC amounted to 2.19. Thus, all MDs were significant. The values of the linear discriminants (LDs) of all pairwise comparisons showed statistically significant differences ($p < 0.0001$ in each case). The sensitivity and the specificity for all comparisons were 1.00. The 100-fold cross-validations and the corresponding cross-validation values (CVVs)[5] were ranging from 59.5 % to 82.0 %: The CVV of the comparison between AATD and COPD was 82.0 %, the CVV of the comparison between AATD and HC was 59.5 %, while the CVV of the comparison between COPD and HC amounted to 80.5 %. Summarized results are displayed in table 2.1.2.

2.2 Pure exhaled breath

A total of 47 volunteers participated in the PEB experiment, 14 AATD patients—including 11 AATD patients in the infusion group—, 23 COPD patients, and 10 healthy controls. The patients of the AATD and the AATD infusion group were partially

[3]The linear discriminant analysis is a statistical method to separate two or more classes.

[4]The Mahalanobis distance is a distance measure, that takes the distribution and variance of the data into account. Statistical significance is achieved with a Mahalanobis distance greater than or equal to 1.96. The formula is given on page 28/1260

[5]The cross-validation value specifies how robust a classification method is, where 100 % is the best value.

Table 2.2.1: Pure exhaled breath participants

	AATD	COPD	HC	AATD infusion
Mean age [a] (standard deviation)	58 (8)	63 (8)	51 (7)	56 (9)
Number ♀ / ♂	3 / 7	5 / 18	7 / 3	3 / 8
Mean %FEV_1 [%] (standard deviation)	41.8 (18.5)	56.5 (19.5)	N/A	44.6 (16.6)
Smoker current / ex	0 / 7	2 / 21	0 / 2	0 / 9

%FEV_1 is defined as the ratio of the actual FEV_1 and the predicted FEV_1.
AATD: α_1-antitrypsin deficiency, COPD: chronic obstructive pulmonary disease, HC: healthy control, FEV_1: forced expiratory volume in one second, N/A: not available.
Data taken from [59].

overlapping. On average, all groups were about the same age, while the HCs (51 years) were slightly younger than COPD patients (63 years) and AATD patients (58 years); the 11 AATD patients in the infusion group were 56 years on average. Not all lung function parameters were available for the HCs. All other groups had comparable %FEV_1 values: COPD (57 %), AATD (42 %), and AATD infusion (45 %). The fraction of females in HC (70 %) was higher than in the other groups, COPD (22 %), AATD (30 %), and AATD infusion (27 %). Only two participants (both COPD patients) were tobacco smokers at the time of sampling. The characteristics are summarized in table 2.2.1. The difference in the %FEV_1 of AATD and COPD patients reached statistical significance ($p = 0.04$). In contrast to the logistic regression analysis, that showed, that the %FEV_1 had no statistically significant impact ($p = 0.08$) regarding the differentiation of COPD and AATD patients.

Considering the three proband groups—AATD, COPD, and HCs—the LDA was able to separate them. Here, the variance of the COPD data was larger than in the EBC experiment (see publication figure 3 (page 30/1262)). Each of the groups formed a separate cluster, that was clearly distinguishable from the others. The corresponding MDs satisfied statistical significance: The MD between AATD and COPD was 2.27, the MD between AATD and HC was 2.67, while the MD between COPD and HC amounted to 2.28. Thus, the MDs were slightly higher than in the EBC experiment. The values of the LDs of all pairwise comparisons showed statistically significant differences (each

Table 2.2.2: Pure exhaled breath results

Group comparison	linear discriminant analysis p-value	CVV	Sensitivity	Specificity	MD
AATD vs COPD	< 0.0001	58.3 %	1.00	1.00	2.27
AATD vs HC	< 0.0001	62.0 %	1.00	1.00	2.67
COPD vs HC	< 0.0001	67.6 %	1.00	1.00	2.28
AATD pre vs post	0.001	53.3 %	1.00	1.00	1.79

Pairwise comparisons and statistical analyses of the linear discriminant analysis data.
CVV: cross-validation value, MD: Mahalanobis distance, AATD: α_1-antitrypsin deficiency, COPD: chronic obstructive pulmonary disease, HC: healthy control, pre: before infusion, post: after infusion.
Data taken from [59].

with $p < 0.0001$). Both, the sensitivity and the specificity for all comparisons were 1.00. The CVVs ranged from 58.3 % to 67.6 %: The CVV of the comparison between AATD and COPD was 58.3 %, the CVV of the comparison between AATD and HC was 62.0 %, while the CVV of the comparison between COPD and HC amounted to 67.6 %. See table 2.2.2 for all details.

Evaluating the data of the infusion experiment, the LDA showed a clear separation between both points in time (each patient was measured before and two hours after the infusion) (see publication figure 4 (page 30/1262)) with a statistically significant difference ($p = 0.001$). Both, the sensitivity and the specificity equaled 1.00. The MD between the points in time equaled 1.79 and did not reach statistical significance. The corresponding CVV of 53.3 % was the lowest value by contrast with the other comparisons. See table 2.2.2 for all details.

2.3 Ion mobility spectrometry

A total of 25 volunteers participated in the IMS experiment, 17 AATD patients—including 2 AATD patients in the infusion group—and 8 COPD patients (data taken from [79]).

Evaluating the data of the infusion experiment, IMS chromatograms differed in at least 22 signals, which had a sum rank (norm U) of 0.000 (see publication table 2 (page 39)).

Considering the two proband groups—AATD and COPD—the IMS chromatograms show 5 discriminating signals, which had a sum rank of less than 0.200 (see publication table 3 (page 42)). A summary of accuracy, sensitivity, specificity, positive and negative predictive values are given in publication table 4 (page 43).

3 Discussion

The results presented in chapter 2 indicate, that the C-320 EN in combination with the described data analysis could distinguish the pattern of VOCs present in the EBC of patients with an AATD from those with a COPD. Furthermore, it could also distinguish between the PEB of AATD and COPD patients. Additionally, the electronic nose also discriminated between patients with either of these diseases from HCs. It might be even possible, to distinguish between AATD patients before and after their weekly body weight-adapted infusion with purified human AAT.

The former suggests, that the VOC patterns differ sufficiently between these two lung diseases to be separated by the C-320 EN, although AATD and COPD share a lot of clinical features. This is supported by the different underlying pathomechanisms (compare sections 1.1.3 and 1.2.3). While COPD is driven by first temporary and then chronic oxidative stress and inflammation, AATD involves a slow but chronic destruction of lung tissue. Furthermore, the latter indicates, that the augmentation of AAT by infusion may lead to a change in the VOC profile of AATD patients.

Although it is unclear, whether the condensation of exhaled air affects the detection of compounds, the results suggest, that EBC contains components that are volatile as well and, therefore, can be recognized by the C-320 EN. The work carried out in this thesis is to the author's knowledge the first published study comparing the smellprints of EBC samples of patients with AATD and COPD.

Other recent studies show, that neither the subjects' age nor the sex do have a statistically significant impact on VOCs detectable by the C-320 EN [43, 118]. See section 3.1 for further details.

As mentioned in the introduction (see section 1.4), the C-320 EN does not allow the identification of single components in a complex VOC sample as it uses cross-reactive sensors. Cross-reactive means here, that both a single sensor responds to multiple substances and one substance is recognized by several sensors [2]. However, the response of the sensor array to a certain VOC sample is unique and results in a so-called "smellprint", a distinct pattern (profile) of this particular sample [137]. The C-320 EN is able to detect differences of VOC mixtures, though, the exact differences can not be determined. This can be done with other devices, that use, for instance, IMS (this is discussed in section 3.2).

The findings of preceding experiments, where multiple C-320 ENs were tested on various sampling setups from Leipzig, München, and Marburg [78], showed, that the Leipzig setup exhibited a relatively high variance suggesting to be less robust than the

München or the Marburg setup to sample human breath. Since, there were no large differences between the München and the Marburg setup, the Marburg setup was the method of choice, as it was established and optimized on site [78].

Furthermore, the results from the preceding experiment also suggest, that the use of one sampling setup across multiple sites and ENs might be limited, since the variance of the ENs could exceed the variance of the probands [78]. This could be the result of slightly different manufacturing, arranging and assembling of single sensors or other component parts. The devices might yield different results due to different years of construction or wear and tear of different construction parts.

The relatively high CVVs in both experiments suggest, that the LDA works well on these kinds of samples. Ideal values of 1.00 are not achieved, but this is not unusual and shows, that the training models for the prediction differ more or less from the model built from the original sample set. The smaller CVVs of the PEB analysis could be attributed to the fact, that these samples had a larger variance than the EBC samples. A major difference in comparing PEB and EBC is, that the additional steps of collecting and stabilizing (degassing) the samples have to be performed in the latter approach. Furthermore, there is a time delay before the sample can be measured due to the additional steps, in which components of the sample may be degrading.

The results provide evidence, that patients with COPD, severe AATD (Z-type), and nonsmoking HCs feature different exhaled smellprints, possibly due to different types of (systemic) inflammation in COPD and AATD patients. This is supported by studies, that have reported differences in airway inflammation between AATD and COPD. Comparing the fraction of exhaled nitric oxide (F_{eNO}) in AATD patients to the F_{eNO} in COPD patients controversial results were found: Higher levels [93] in contrast to lower levels [90] of F_{eNO} could be measured. Neutrophil chemotactic activity in sputum appears to be significantly higher in patients with AATD than in patients with COPD [157]. It is very likely, that the specific defect in AATD has a multitude of cellular and biochemical consequences, including pulmonary and systemic aspects [1, 21, 28, 39, 48, 68, 86, 87, 88, 108, 109, 116, 119].

Regarding the data from AATD patients before and after treatment with purified human AAT, it can be speculated, that the given AAT leads to a change in the VOC profile. It has been demonstrated, that AAT is directly measurable in the bronchoalveolar lavage (BAL) fluid after this treatment [64]. Thus, it is likely, that the treatment with AAT results in modifications of the exhaled VOC profile, and has local and systemic effects. This is supported by the aforementioned evidence of biological effects of

the infusion therapy [32]. Though, an inflammatory response after the augmentation, caused by the administration of foreign human proteins, cannot be excluded [130].

The usage of EBC and the C-320 EN is a cost-effective option to assess the lung, as it is noninvasive, reproducible, simple, and only needs a few cheap disposable items while in service.

In conclusion, the C-320 EN combined with the applied analyses could cost-efficiently detect differences in the smellprints of patients with COPD and AATD. The AAT infusion therapy seems to change the VOC patterns of affected individuals. The first suggests, that an electronic nose could be helpful in the assessment of AATD. Hence, the use of the C-320 EN in the diagnosis of AATD should be evaluated further. Both methods, assessment of exhaled breath by an EN and by EBC, could be used in larger trials, as they are noninvasive and sampling is simple to perform.

3.1 Analyses

There are many adequate algorithms for analyzing these types of data, each with limitations [158]. For example, methods like support vector machines (SVMs) or hidden Markov models (HMMs) might be favorable to separate groups, but these methods are often very abstract, difficult to compute, and hard to visualize. Other, unsupervised methods, such as hierarchical clustering, might include additional parameters to reveal possible subgroups [42]. Clustering strongly depends on initiation, metric, number of clusters, etc. At the same time, pitfalls lurk by misapplying certain methods, such as the usage of orthogonal functions on nonindependent data. Furthermore, the suitability of the different methods might vary greatly depending on the investigated diseases or problems. Pioneering work mostly requires simple data models and good visualizability. Supported by the high values of sensitivity and specificity, the LDA seemed to be a good choice for the tasks at hand.

However, the important limitation to these discussed studies was the relatively low number of participants per study group. Small sample sizes can increase the risk of overfitting, especially if the samples include many variables. Lower CVVs may also indicate overfitting, but can also be the result of small sample sizes, as single outliers have increased impact on small sample sets. Therefore, it is crucial, that the given samples are well-defined, low-noise, and representative. Although, it is still common to categorize COPD patients spirometrically via airflow limitation, this grouping might lead to improperly defined classes. Spirometry guidelines demand deviations of FEV_1 up to 150 ml [105]. As spirometric assessment depends on the

proband's adherence and form of the day [105], deviation of FEV_1 range from 50 ml to 60 ml, in individual cases more than 100 ml [57]. Annual decline of FEV_1 for HCs (about 30 ml to 40 ml [46]), nonsmoking (about 40 ml to 50 ml) and smoking (about 70 ml) AATD patients [121], and COPD patients (about 30 ml to 80 ml [76]) range within the maximal permitted deviation. Hence, FEV_1 *per se* might be not precise enough, to allow a proper classification of probands. Thus, the groups of COPD and AATD patients could be more heterogeneous than assumed, having led to low CVVs as a result of lacking well-defined proband groups.

As mentioned earlier, other studies show, that the potential confounders age or sex have no influence on EB assessed via an EN [43, 118]. At the same time, differences in the FEV_1 between AAT-deficient and COPD patients did not affect results and conclusions as shown in sections 2.1 and 2.2. Although the differences in the FEV_1 between AAT-deficient and COPD patients of the PEB experiment were statistically significant, the logistic regression revealed, that despite of these differences, the FEV_1 had no statistically significant impact.

3.2 Outlook

Sampling could be improved by preconcentrating the samples and reducing possible contamination sources, like perfumes or other persons, that are present.

Beyond that, building a database containing smellprints of several well-defined patient groups could help to extend diagnostic purposes and to cover a comprehensive range of diseases. Especially areas, where diagnoses are difficult to make, were explored based on this work. These may include neurology and pediatrics, where it can be very challenging to perform required biopsies [5, 127], and sleep medicine, which may demand extensive examinations [53, 54]. Furthermore, it was shown, that EN technology can be integrated into daily clinical practice [30].

Other researchers have built ENs with promising custom-made sensors, for instance, based on gold nanoparticles [17]. Some of them examined the VOCs in the headspace of human lung cell line cultures, but also in human breath, and can distinguish between VOCs of healthy persons and such with cancer. In addition, it is possible to differentiate between certain types of lung cancer [9, 118]. Furthermore, novel nickel-based chemi-sensors promise faster recovery rates during purging and less sensitivity to water [56].

Other techniques

Devices, that extend the EN techniques, provide promising but cost-intensive possibilities for diagnostic examinations. The BIONOTE device, which is also able to analyze the liquid phase and optical properties, is able to support noninvasively lung cancer screening [126].

Preliminary results of the comparison of EB from patients with COPD and AATD with the BioScout IMS device showed, that these two patient groups can also be differentiated by the IMS (see section 2.3). This way, both these diseases can be distinguished by single VOCs in EB, that are most likely *n*-Butanol, Butanone, 2-Propanol, and 2-Hexanone [79].

A similar technique is proton-transfer-reaction mass spectrometer (PTR-MS). This method could determine various VOCs in EB associated with liver cirrhosis [44].

Additional knowledge could be gained by determining the composition of the VOC mixtures and to identify single compounds that differ in certain lung disorders, although the described methods already allow a distinction without knowing exact details. For this reason, more detailed data about differentiating compounds could help to gain important insight into diseases and to investigate further treatment possibilities. With the help of newer devices and techniques, such as the PTR-MS or IMS coupled with GC/MS, this is very likely to be solved in the near future.

Publication list

Several issues discussed in section 3 have been explored in further projects leading to the following publications in peer-reviewed journals:

1. **A. Hattesohl**, R. A. Jörres, H. Dressler, S. Schmid, C. Vogelmeier, T. Greulich, S. Noeske, R. Bals, and A.-R. Koczulla:
   Discrimination between COPD patients with and without α_1-antitrypsin deficiency using an electronic nose, *Respirology* **16** (2011), no. 16, 1258–1264

2. A.-R. Koczulla, **A. Hattesohl**, S. Schmid, B. Bödeker, S. Maddula, and J. I. Baumbach:
   MCC/IMS as potential noninvasive technique in the diagnosis of patients with COPD with and without alpha 1-antitrypsin deficiency, *Int J Ion Mob Spec* **14** (2011), no. 4, 177–185

3. A.-R. Koczulla, **A. Hattesohl**, H. Biller, J. Hofbauer, J. Hohlfeld, C. Oeser, H. Wirtz, and R. A. Jörres:
   [Comparison of four identical electronic noses and three measurement set-ups], *Pneumologie* **65** (2011), no. 8, 465–470 (German)

4. A.-R. Koczulla, **A. Hattesohl**, H. Biller, J. Hofbauer, J. Hohlfeld, C. Oeser, C. Gessner, C. Vogelmeier, J. I. Baumbach, H. Wirtz, and R. A. Jörres:
   [Smelling diseases? A short review on electronic noses], *Pneumologie* **65** (2011), no. 7, 401–405 (German)

5. T. Greulich, **A. Hattesohl**, A. Grabisch, J. Koepke, S. Schmid, S. Noeske, C. Nell, M. Wencker, R. A. Jörres, C. Vogelmeier, U. Köhler, and A.-R. Koczulla:
   Detection of obstructive sleep apnoea by an electronic nose, *Eur Respir J* (2012)

6. J. P. Bach, M. Gold, D. Mengel, **A. Hattesohl**, D. Lubbe, S. Schmid, B. Tackenberg, J. Rieke, S. Maddula, J. I. Baumbach, C. Nell, T. Boeselt, J. Michelis, J. Alferink, M. Heneka, W. Oertel, F. Jessen, S. Janciauskiene, C. Vogelmeier, R. Dodel, A. R. Koczulla:
   Measuring Compounds in Exhaled Air to Detect Alzheimer's Disease and Parkinson's Disease, *PLoS One* **10** (2015), no. 7, e0132227

7. E.-M. Hüttmann, T. Greulich, **A. Hattesohl**, S. Schmid, S. Noeske, C. Herr, G. John, R. A. Jörres, B. Müller, C. Vogelmeier, and A.-R. Koczulla:
   Comparison of two devices and two breathing patterns for exhaled breath condensate sampling, *PLoS ONE* **6** (2011), no. 11, e27467

8. E.-M. Hüttmann, T. Greulich, **A. Hattesohl**, S. Schmid, S. Noeske, C. Herr, G. John, R. A. Jörres, B. Müller, C. Vogelmeier, and A.-R. Koczulla:
   Correction: Comparison of two devices and two breathing patterns for exhaled breath condensate sampling, *PLoS ONE* **11** (2016), no. 3, e0152620

9. S. T. Schmid, J. Koepke, M. Dresel, **A. Hattesohl**, E. Frenzel, J. Pérez, D. A. Lomas, E. Miranda, T. Greulich, S. Noeske, C. Vogelmeier, S. M. Janciauskiene, and A.-R. Koczulla:
   The effects of weekly augmentation therapy in patients with PiZZ α1-antitrypsin deficiency, *Int J Chron Obstruct Pulmon Dis* **7** (2012), 687–696

10. T. Rogosch, N. Herrmann, R. F. Maier, E. Domann, **A. Hattesohl**, A.-R. Koczulla, and M. Zemlin:
DETECTION OF BLOODSTREAM INFECTIONS AND PREDICTION OF BRONCHOPULMONARY DYSPLASIA IN PRETERM NEONATES WITH AN ELECTRONIC NOSE, *J Pediatr* **165** (2014), no. 3, 622–624

11. T. Zakharkina, A.-R. Koczulla, O. Mardanova, **A. Hattesohl**, and R. Bals:
DETECTION OF MICROORGANISMS IN EXHALED BREATH CONDENSATE DURING ACUTE EXACERBATIONS OF COPD, *Respirology* **16** (2011), no. 6, 932–938

List of tables

References

[1] S. Alam, Z. Li, S. Janciauskiene, and R. Mahadeva. Oxidation of Z α_1-antitrypsin by cigarette smoke induces polymerization: a novel mechanism of early-onset emphysema. *Am J Respir Cell Mol Biol*, 45(2):261–269, Aug 2011. (Cited on pages 4 and 16)

[2] K. J. Albert, N. S. Lewis, C. L. Schauer, et al. Cross-reactive chemical sensor arrays. *Chem Rev*, 100(7):2595–2626, Jul 2000. (Cited on page 15)

[3] Alpha-1-Antitrypsin Deficiency Registry Study Group. Survival and FEV_1 decline in individuals with severe deficiency of α_1-antitrypsin. *Am J Respir Crit Care Med*, 158(1):49–59, Jul 1998. (Cited on page 5)

[4] B & S Analytik GmbH. BioScout, Dortmund, Germany. `http://www.bs-analytik.de`. (Cited on page 7)

[5] J.-P. Bach, M. Gold, D. Mengel, et al. Measuring compounds in exhaled air to detect alzheimer's disease and parkinson's disease. *PLoS One*, 10(7):e0132227, 2015. (Cited on page 18)

[6] B. Balint, S. A. Kharitonov, T. Hanazawa, et al. Increased nitrotyrosine in exhaled breath condensate in cystic fibrosis. *Eur Respir J*, 17(6):1201–1207, Jun 2001. (Cited on page 6)

[7] R. Bals. Alpha-1-antitrypsin deficiency. *Best Pract Res Clin Gastroenterol*, 24(5):629–633, Oct 2010. (Cited on page 3)

[8] R. Bals, A.-R. Koczulla, V. Kotke, et al. Identification of individuals with alpha 1-antitrypsin deficiency by a targeted screening program. *Respir Med*, 101(8):1708–1714, Aug 2007. (Cited on pages 1, 3, and 4)

[9] O. Barash, N. Peled, U. Tisch, et al. Classification of lung cancer histology by gold nanoparticle sensors. *Nanomedicine*, 8(5):580–589, Jul 2012. (Cited on page 18)

[10] P. J. Barnes, S. D. Shapiro, and R. A. Pauwels. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *Eur Respir J*, 22(4):672–688, Oct 2003. (Cited on page 2)

[11] J. I. Baumbach. Ion mobility spectrometry coupled with multi-capillary columns for metabolic profiling of human breath. *J Breath Res*, 3(3):034001, Sep 2009. (Cited on page 7)

[12] A. Belaaouaj, K. S. Kim, and S. D. Shapiro. Degradation of outer membrane protein A in *Escherichia coli* killing by neutrophil elastase. *Science*, 289(5482):1185–1188, Aug 2000. (Cited on page 3)

[13] A. Belaaouaj, R. McCarthy, M. Baumann, et al. Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. *Nat Med*, 4(5):615–618, May 1998. (Cited on page 3)

[14] F. Beske, A. Katalinic, E. Peters, and R. Pritzkuleit. *Morbiditätsprognose 2050: Ausgewählte Krankheiten*

für Deutschland, Brandenburg und Schleswig-Holstein, volume 114 of *Schriftenreihe*. Schmidt & Klaunig, 2009. (Cited on page 1)

[15] I. Blanco, F. J. de Serres, E. Fernández-Bustillo, et al. Estimated numbers and prevalence of PI*S and PI*Z alleles of α_1-antitrypsin deficiency in European countries. *Eur Respir J*, 27(1):77–84, Jan 2006. (Cited on page 3)

[16] E. Bogen. The diagnosis of drunkenness—A quantitative study of acute alcoholic intoxication. *Cal West Med*, 26(6):778–783, Jun 1927. (Cited on page 5)

[17] M. Brust and C. J. Kiely. Some recent advances in nanostructure preparation from gold and silver particles: a short topical review. *Colloids Surf A*, 202(2–3):175–86, 2002. (Cited on page 18)

[18] A. Bunkowski, B. Bödeker, S. Bader, et al. MCC/IMS signals in human breath related to sarcoidosis—results of a feasibility study using an automated peak finding procedure. *J Breath Res*, 3(4):046001, Dec 2009. (Cited on page 7)

[19] R. J. Butland, J. Pang, E. R. Gross, et al. Two-, six-, and 12-minute walking tests in respiratory disease. *Br Med J (Clin Res Ed)*, 284(6329):1607–1608, May 1982. (Cited on page 2)

[20] M. A. Campos, A. Wanner, G. Zhang, and R. A. Sandhaus. Trends in the diagnosis of symptomatic patients with α_1-antitrypsin deficiency between 1968 and 2003. *Chest*, 128(3):1179–1186, Sep 2005. (Cited on pages 1 and 3)

[21] J. A. Carlson, B. B. Rogers, R. N. Sifers, et al. Accumulation of PiZ α_1-antitrypsin causes liver damage in transgenic mice. *J Clin Invest*, 83(4):1183–1190, Apr 1989. (Cited on pages 4 and 16)

[22] G. E. Carpagnano, S. A. Kharitonov, O. Resta, et al. Increased 8-isoprostane and interleukin-6 in breath condensate of obstructive sleep apnea patients. *Chest*, 122(4):1162–1167, Oct 2002. (Cited on page 6)

[23] M. Chan-Yeung, D. A. Enarson, and S. M. Kennedy. The impact of grain dust on respiratory health. *Am Rev Respir Dis*, 145(2 Pt 1):476–487, Feb 1992. (Cited on page 1)

[24] J. Cichy, J. Potempa, and J. Travis. Biosynthesis of α_1-proteinase inhibitor by human lung-derived epithelial cells. *J Biol Chem*, 272(13):8250–8255, Mar 1997. (Cited on page 2)

[25] A. B. Cohen. Interrelationships between the human alveolar macrophage and alpha-1-antitrypsin. *J Clin Invest*, 52(11):2793–2799, Nov 1973. (Cited on page 2)

[26] J. Collins and E. J. Stern. *Chest Radiology: The Essentials*. Wolters Kluwer Health/Lippincott Williams & Wilkins, 2nd edition, 2008. (Cited on page 2)

[27] C. S. Creaser, J. R. Griffiths, C. J. Bramwell, et al. Ion mobility spectrometry: a review. Part 1. Structural analysis by mobility measurement. *Analyst*, 129:984–994, 2004. (Cited on page 7)

[28] R. G. Crystal. The α_1-antitrypsin gene and its deficiency states. *Trends Genet*, 5(12):411–417, Dec 1989. (Cited on pages 2, 4, and 16)

[29] F. J. de Serres. Worldwide racial and ethnic distribution of α_1-antitrypsin deficiency: summary of an analysis of published genetic epidemiologic surveys. *Chest*, 122(5):1818–1829, Nov 2002. (Cited on page 3)

[30] R. de Vries, P. Brinkman, M. P. van der Schee, et al. Integration of electronic nose technology with spirometry: validation of a new approach for exhaled breath analysis. *J Breath Res*, 9(4):046001, Oct 2015. (Cited on page 18)

[31] D. L. DeMeo and E. K. Silverman. α_1-antitrypsin deficiency · 2: genetic aspects of α_1-antitrypsin deficiency: phenotypes and genetic modifiers of emphysema risk. *Thorax*, 59(3):259–264, Mar 2004. (Cited on page 3)

[32] J. A. Dickens and D. A. Lomas. Why has it been so difficult to prove the efficacy of alpha-1-antitrypsin replacement therapy? Insights from the study of disease pathogenesis. *Drug Des Devel Ther*, 5:391–405, 2011. (Cited on pages 5 and 17)

[33] A. Dirksen, E. Piitulainen, D. G. Parr, et al. Exploring the role of CT densitometry: a randomised study of augmentation therapy in α_1-antitrypsin deficiency. *Eur Respir J*, 33(6):1345–1353, Jun 2009. (Cited on page 5)

[34] S. Dragonieri, J. T. Annema, R. Schot, et al. An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. *Lung Cancer*, 64(2):166–170, May 2009. (Cited on page 6)

[35] S. Dragonieri, R. Schot, B. J. A. Mertens, et al. An electronic nose in the discrimination of patients with asthma and controls. *J Allergy Clin Immunol*, 120(4):856–862, Oct 2007. (Cited on page 6)

[36] R. Dutta, E. L. Hines, J. W. Gardner, and P. Boilot. Bacteria classification using Cyranose 320 electronic nose. *BioMed Eng Online*, 1:4, Oct 2002. (Cited on page 6)

[37] R. M. Effros, K. W. Hoagland, M. Bosbous, et al. Dilution of respiratory solutes in exhaled condensates. *Am J Respir Crit Care Med*, 165(5):663–669, Mar 2002. (Cited on page 6)

[38] M. S. Eledrisi, M. S. Alshanti, M. F. Shah, et al. Overview of the diagnosis and management of diabetic ketoacidosis. *Am J Med Sci*, 331(5):243–251, May 2006. (Cited on page 5)

[39] P. R. Elliott, D. Bilton, and D. A. Lomas. Lung polymers in Z α_1-antitrypsin deficiency-related emphysema. *Am J Respir Cell Mol Biol*, 18(5):670–674, May 1998. (Cited on pages 4 and 16)

[40] S. Fähndrich, C. Herr, T. Greulich, et al. Sex differences in alpha-1-antitrypsin deficiency lung disease–analysis from the german registry. *COPD*, 12 Suppl 1:58–62, May 2015 (eng). (Cited on page 3)

[41] Federal Statistical Office, branch Bonn. Statistics on the causes of death: Number of deaths. Classification: years, region, age, sex, family status, ICD-10, `www.gbe-bund.de` (retrieved 19 Nov, 2012), 2011. (Cited on page 1)

[42] N. Fens, A. G. J. van Rossum, P. Zanen, et al. Subphenotypes of mild-to-moderate COPD by factor and cluster analysis of pulmonary function, CT imaging and breathomics in a population-based survey. *COPD*, 10(3):277–285, Jun 2013. (Cited on page 17)

[43] N. Fens, A. H. Zwinderman, M. P. van der Schee, et al. Exhaled breath profiling enables discrimination of chronic obstructive pulmonary disease and asthma. *Am J Respir Crit Care Med*, 180(11):1076–1082, Dec 2009. (Cited on pages 6, 15, and 18)

[44] R. Fernández Del Río, M. E. O'Hara, A. Holt, et al. Volatile biomarkers in breath associated with liver cirrhosis – comparisons of pre- and post-liver transplant breath samples. *EBioMedicine*, 2(9):1243–1250, Sep 2015. (Cited on page 19)

[45] C. Fischer. *The Corpus: The Hippocratic Writings*. Kaplan Classics of Medicine. Kaplan Publishing, original edition, 2008. (Cited on page 5)

[46] C. Fletcher and R. Peto. The natural history of chronic airflow obstruction. *Br Med J*, 1(6077):1645–1648, Jun 1977. (Cited on page 18)

[47] C. M. Fletcher, P. C. Elmes, A. S. Fairbairn, and C. H. Wood. The significance of respiratory symptoms and the diagnosis of chronic bronchitis in a working population. *Br Med J*, 2(5147):257–266, Aug 1959. (Cited on page 2)

[48] J. E. Gadek, G. A. Fells, R. L. Zimmerman, et al. Antielastases of the human alveolar structures. Implications for the protease-antiprotease theory of emphysema. *J Clin Invest*, 68(4):889–898, Oct 1981. (Cited on pages 4 and 16)

[49] C. E. Garner, S. Smith, P. K. Bardhan, et al. A pilot study of faecal volatile organic compounds in faeces from cholera patients in Bangladesh to determine their utility in disease diagnosis. *Trans R Soc Trop Med Hyg*, 103(11):1171–1173, Nov 2009. (Cited on page 5)

[50] C. Gessner, S. Hammerschmidt, H. Kuhn, et al. Breath condensate nitrite correlates with hyperinflation in chronic obstructive pulmonary disease. *Respir Med*, 101(11):2271–2278, Nov 2007. (Cited on page 6)

[51] Global Initiative for Chronic Obstructive Lung Disease (GOLD), Inc. *Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease, updated 2015*, Jan 2015. (Cited on pages 1, 2, 3, and 4)

[52] S. M. Gordon, J. P. Szidon, B. K. Krotoszynski, et al. Volatile organic compounds in exhaled air from patients with lung cancer. *Clin Chem*, 31(8):1278–1282, Aug 1985. (Cited on page 5)

[53] A. Grabisch, T. Greulich, A. Hattesohl, et al. Die elektronische Nase (Cyranose 320) kann zwischen Patienten mit obstruktivem Schlafapnoe-Syndrom und gesunden Kontrollen unterscheiden. *Pneumologie*, 65(S 01):V323, 2011 (German). (Cited on page 18)

[54] T. Greulich, A. Hattesohl, A. Grabisch, et al. Detection of obstructive sleep apnoea by an electronic nose. *Eur Respir J*, 42(1):145–155, Jul 2013. (Cited on page 18)

[55] T. Greulich and C. F. Vogelmeier. Alpha-1-antitrypsin deficiency: increasing awareness and improving diagnosis. *Ther Adv Respir Dis*, Sep 2015. (Cited on page 3)

[56] P. J. W. Hands, P. J. Laughlin, and D. Bloor. Metal–polymer composite sensors for volatile organic compounds: part 1. Flow-through chemi-resistors. *Sens Actuators, B*, 162(1):400–408, 2012. (Cited on page 18)

[57] J. L. Hankinson and K. M. Bang. Acceptability and reproducibility criteria of the American Thoracic Society as observed in a sample of the general population. *Am Rev Respir Dis*, 143(3):516–521, Mar 1991. (Cited on page 18)

[58] R. N. Harger, E. B. Lamb, and H. R. Hulpieu. A rapid chemical test for intoxication employing breath: a new reagent for alcohol and a procedure for estimating the concentration of alcohol in the body from the ratio of alcohol to carbon dioxide in the breath. *JAMA*, 110(11):779–785, Mar 1938. (Cited on page 5)

[59] A. Hattesohl, R. A. Jörres, H. Dressel, et al. Discrimination between COPD patients with and without alpha 1-antitrypsin deficiency using an electronic nose. *Respirology*, 16(8):1258–1264, Nov 2011. (Cited on pages 1, 5, 6, 9, 10, 11, 12, and 37)

[60] A. T. Hill, E. J. Campbell, D. L. Bayley, et al. Evidence for excessive bronchial inflammation during an acute exacerbation of chronic obstructive pulmonary disease in patients with α_1-antitrypsin deficiency (PiZ). *Am J Respir Crit Care Med*, 160(6):1968–1975, Dec 1999. (Cited on page 3)

[61] J. C. Hogg. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet*, 364(9435):709–721, Aug 2004. (Cited on page 2)

[62] J. M. Hood, L. J. Koep, R. L. Peters, et al. Liver transplantation for advanced liver disease with alpha-1-antitrypsin deficiency. *N Engl J Med*, 302(5):272–275, Jan 1980. (Cited on page 2)

[63] I. Horváth, J. Hunt, P. J. Barnes, et al. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J*, 26(3):523–548, Sep 2005. (Cited on page 6)

[64] R. C. Hubbard, N. G. McElvaney, S. E. Sellers, et al. Recombinant DNA–produced α1-antitrypsin administered by aerosol augments lower respiratory tract antineutrophil elastase defenses in individuals with α1-antitrypsin deficiency. *J Clin Invest*, 84(4):1349–1354, Oct 1989. (Cited on page 16)

[65] E.-M. Hüttmann, T. Greulich, A. Hattesohl, et al. Comparison of two devices and two breathing patterns for exhaled breath condensate sampling. *PLoS ONE*, 6(11):e27467, 11 2011.

[66] E.-M. Hüttmann, T. Greulich, J. Koepke, et al. Correction: Comparison of two devices and two breathing patterns for exhaled breath condensate sampling. *PLoS ONE*, 11(3):e0152620, Mar 2016.

[67] Intelligent Optical Systems, Inc. Cyranose 320, 2520 W. 237th Street, Torrance, CA 90505-5217. `http://www.intopsys.com`, `http://www.sensigent.com/products/cyranose.html`. (Cited on page 6)

[68] S. M. Janciauskiene, R. Bals, A.-R. Koczulla, et al. The discovery of α_1-antitrypsin and its role in health and disease. *Respir Med*, 105(8):1129–1139, Aug 2011. (Cited on pages 1, 2, 3, 4, and 16)

[69] E. D. Janus, N. T. Phillips, and R. W. Carrell. Smoking, lung function, and α_1-antitrypsin deficiency. *Lancet*, 1(8421):152–154, Jan 1985. (Cited on page 3)

[70] J. O. Jeppsson. Amino acid substitution glu→lys in α_1-antitrypsin piz. *FEBS Lett*, 65(2):195–197, Jun 1976. (Cited on page 3)

[71] P. W. Jones, G. Harding, P. Berry, et al. Development and first validation of the COPD Assessment Test. *Eur Respir J*, 34(3):648–654, Sep 2009. (Cited on page 2)

[72] P. W. Jones, F. H. Quirk, C. M. Baveystock, and P. Littlejohns. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. *Am Rev Respir Dis*, 145(6):1321–1327, Jun 1992. (Cited on page 2)

[73] A. Karakatsani, S. Andreadaki, K. Katsouyanni, et al. Air pollution in relation to manifestations of chronic pulmonary disease: a nested case-control study in Athens, Greece. *Eur J Epidemiol*, 18(1):45–53, Jan 2003. (Cited on page 1)

[74] K. Kawabata, T. Hagio, and S. Matsuoka. The role of neutrophil elastase in acute lung injury. *Eur J Pharmacol*, 451(1):1–10, Sep 2002. (Cited on page 3)

[75] S. M. Kennedy, H. Dimich-Ward, A. Desjardins, et al. Respiratory health among retired grain elevator workers. *Am J Respir Crit Care Med*, 150(1):59–65, Jul 1994. (Cited on page 1)

[76] H. A. Kerstjens, P. L. Brand, and D. S. Postma. Risk factors for accelerated decline among patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, 154(6 Pt 2):S266–S272, Dec 1996. (Cited on page 18)

[77] A.-R. Koczulla, A. Hattesohl, H. Biller, et al. [Smelling diseases? A short review on electronic noses]. *Pneumologie*, 65(7):401–405, Jul 2011 (German).

[78] A.-R. Koczulla, A. Hattesohl, H. Biller, et al. [Comparison of four identical electronic noses and three measurement set-ups]. *Pneumologie*, 65(8):465–470, Aug 2011 (German). (Cited on pages 15 and 16)

[79] A.-R. Koczulla, A. Hattesohl, S. Schmid, et al. MCC/IMS as potential noninvasive technique in the diagnosis of patients with COPD with and without alpha 1-antitrypsin deficiency. *Int J Ion Mob Spec*, 14(4):177–185, Dec 2011. (Cited on pages 5, 12, 19, and 47)

[80] A.-R. Koczulla, S. Noeske, C. Herr, et al. Acute and chronic effects of smoking on inflammation markers in exhaled breath condensate in current smokers. *Respiration*, 79(1):61–67, 2010. (Cited on page 6)

[81] A.-R. Koczulla, S. Noeske, C. Herr, et al. Alpha-1 antitrypsin is elevated in exhaled breath condensate and serum in exacerbated COPD patients. *Respir Med*, 106(1):120–126, Jan 2012. (Cited on page 6)

[82] F. Kueppers. Alpha-1-antitrypsin: physiology, genetics and pathology. *Humangenetik*, 11(3):177–189, 1971. (Cited on page 2)

[83] S. Y. Lai, O. F. Deffenderfer, W. Hanson, et al. Identification of upper respiratory bacterial pathogens with the electronic nose. *Laryngoscope*, 112(6):975–979, Jun 2002. (Cited on page 6)

[84] C. Larsson. Natural history and life expectancy in severe alpha$_1$-antitrypsin deficiency, Pi Z. *Acta Med Scand*, 204(5):345–351, 1978. (Cited on page 3)

[85] N. S. Lewis. Comparisons between mammalian and artificial olfaction based on arrays of carbon black-polymer composite vapor detectors. *Acc Chem Res*, 37(9):663–672, Sep 2004. (Cited on page 6)

[86] D. A. Lomas, P. R. Elliott, S. K. Sidhar, et al. α_1-antitrypsin Mmalton (Phe^{52}-deleted) forms loop-sheet polymers in vivo. Evidence for the C sheet mechanism of polymerization. *J Biol Chem*, 270(28):16864–16870, Jul 1995. (Cited on pages 4 and 16)

[87] D. A. Lomas, D. L. Evans, S. R. Stone, et al. Effect of the Z mutation on the physical and inhibitory properties of α_1-antitrypsin. *Biochemistry*, 32(2):500–508, Jan 1993. (Cited on pages 4 and 16)

[88] D. A. Lomas, J. T. Finch, K. Seyama, et al. α_1-antitrypsin S_{iiyama} (Ser^{53}→Phe). Further evidence for intracellular loop-sheet polymerization. *J Biol Chem*, 268(21):15333–15335, Jul 1993. (Cited on pages 4 and 16)

[89] A. Lukacs, H. C. Korting, G. Ruckdeschel, and W. Ehret. Do cutaneous coryneform bacteria produce short-chain fatty acids in vitro? *Dermatologica*, 182(1):32–34, 1991. (Cited on page 5)

[90] R. F. Machado, J. K. Stoller, D. Laskowski, et al. Low levels of nitric oxide and carbon monoxide in α_1-antitrypsin deficiency. *J Appl Physiol*, 93(6):2038–2043, Dec 2002. (Cited on page 16)

[91] A. M. Mahoney, B. J. Weetjens, C. Cox, et al. Using giant African pouched rats to detect tuberculosis in human sputum samples: 2010 findings. *Pan Afr Med J*, 9:28, 2011. (Cited on page 5)

[92] E. Majewska, M. Kasielski, R. Luczynski, et al. Elevated exhalation of hydrogen peroxide and thiobarbituric acid reactive substances in patients with community acquired pneumonia. *Respir Med*, 98(7):669–676, Jul 2004. (Cited on page 6)

[93] M. Malerba, B. Ragnoli, and A. Radaeli. Exhaled nitric oxide levels in alpha-1-antitrypsin PiMZ subjects. *J Intern Med*, 265(3):382–387, Mar 2009. (Cited on page 16)

[94] B. Malnic, J. Hirono, T. Sato, and L. B. Buck. Combinatorial receptor codes for odors. *Cell*, 96(5):713–723, Mar 1999. (Cited on page 5)

[95] D. M. Mannino and A. S. Buist. Global burden of COPD: risk factors, prevalence, and future trends. *Lancet*, 370(9589):765–773, Sep 2007. (Cited on page 1)

[96] E. M. Marek, J. Volke, I. Hawener, et al. Measurements of lactate in exhaled breath condensate at rest and after maximal exercise in young and healthy subjects. *J Breath Res*, 4(1):017105, Jan 2010. (Cited on page 6)

[97] J. M. Mateo. Kin-recognition abilities and nepotism as a function of sociality. *Proc Biol Sci*, 269(1492):721–727, Apr 2002. (Cited on page 5)

[98] C. D. Mathers and D. Loncar. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*, 3(11):e442, Nov 2006. (Cited on page 1)

[99] M. McCulloch, T. Jezierski, M. Broffman, et al. Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. *Integr Cancer Ther*, 5(1):30–39, Mar 2006. (Cited on pages 1 and 5)

[100] N. G. McElvaney, J. K. Stoller, A. S. Buist, et al. Baseline characteristics of enrollees in the National Heart, Lung and Blood Institute Registry of α_1-antitrypsin deficiency. *Chest*, 111(2):394–403, Feb 1997. (Cited on pages 1, 3, and 4)

[101] A. M. B. Menezes, P. C. Hallal, R. Perez-Padilla, et al. Tuberculosis and airflow obstruction: evidence from the PLATINO study in Latin America. *Eur Respir J*, 30(6):1180–1185, Dec 2007. (Cited on page 1)

[102] A. M. B. Menezes, R. Perez-Padilla, J. R. B. Jardim, et al. Chronic obstructive pulmonary disease in five Latin American cities (the PLATINO study): a prevalence study. *Lancet*, 366(9500):1875–1881, Nov 2005. (Cited on page 1)

[103] J. H. Menkes, P. L. Hurst, and J. M. Craig. A new syndrome: progressive familial infantile cerebral dysfunction associated with an unusual urinary substance. *Pediatrics*, 14(5):462–467, Nov 1954. (Cited on page 5)

[104] G. F. Mgode, B. J. Weetjens, T. Nawrath, et al. Diagnosis of tuberculosis by trained African giant pouched rats and confounding impact of pathogens and microflora of the respiratory tract. *J Clin Microbiol*, 50(2):274–280, Feb 2012. (Cited on page 5)

[105] M. R. Miller, J. Hankinson, V. Brusasco, et al. Standardisation of spirometry. *Eur Respir J*, 26(2):319–338, Aug 2005. (Cited on pages 17 and 18)

[106] P. Montuschi and P. J. Barnes. Exhaled leukotrienes and prostaglandins in asthma. *J Allergy Clin Immunol*, 109(4):615–620, Apr 2002. (Cited on page 6)

[107] P. Montuschi, S. Martello, M. Felli, et al. Liquid chromatography/mass spectrometry analysis of exhaled leukotriene B_4 in asthmatic children. *Respir Res*, 6:119, 2005. (Cited on page 6)

[108] A. T. Mulgrew, C. C. Taggart, M. W. Lawless, et al. Z α_1-antitrypsin polymerizes in the lung and acts as a neutrophil chemoattractant. *Chest*, 125(5):1952–1957, May 2004. (Cited on pages 4 and 16)

[109] M. Needham and R. A. Stockley. α_1-antitrypsin deficiency · 3: clinical manifestations and natural history. *Thorax*, 59(5):441–445, May 2004. (Cited on pages 4 and 16)

[110] M. Nencki. Ueber das Vorkommen von Methylmercaptan im menschlichen Harn nach Spargelgenuss. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 28(3–4):206–209, Apr 1891 (German). (Cited on page 5)

[111] K. Nishimura, T. Izumi, M. Tsukino, and T. Oga. Dyspnea is a better predictor of 5-year survival than airway obstruction in patients with COPD. *Chest*, 121(5):1434–1440, May 2002. (Cited on page 2)

[112] D. L. Nolte, J. R. Mason, G. Epple, et al. Why are predator urines aversive to prey? *J Chem Ecol*, 20(7):1505–1516, Jul 1994. 10.1007/BF02059876. (Cited on page 5)

[113] M. E. O'Brien, K. Pennycooke, T. P. Carroll, et al. The impact of smoke exposure on the clinical phenotype of alpha-1 antitrypsin deficiency in ireland: exploiting a national registry to understand a rare disease. *COPD*, 12 Suppl 1:2–9, May 2015 (eng). (Cited on page 3)

[114] M. C. Owen, R. W. Carrell, and S. O. Brennan. The abnormality of the s variant of human α-1-antitrypsin. *Biochim Biophys Acta*, 453(1):257–261, Nov 1976. (Cited on page 3)

[115] P. Pääkkö, M. Kirby, R. M. du Bois, et al. Activated neutrophils secrete stored α_1-antitrypsin. *Am J Respir Crit Care Med*, 154(6 Pt 1):1829–1833, Dec 1996. (Cited on page 2)

[116] J. S. Parmar, R. Mahadeva, B. J. Reed, et al. Polymers of α_1-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. *Am J Respir Cell Mol Biol*, 26(6):723–730, Jun 2002. (Cited on pages 4 and 16)

[117] L. Pauling, A. B. Robinson, R. Teranishi, and P. Cary. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. *Proc Natl Acad Sci U S A*, 68(10):2374–2376, Oct 1971. (Cited on page 6)

[118] G. Peng, M. Hakim, Y. Y. Broza, et al. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. *Br J Cancer*, 103(4):542–551, Aug 2010. (Cited on pages 15 and 18)

[119] D. H. Perlmutter. Liver injury in α_1-antitrypsin deficiency: an aggregated protein induces mitochondrial injury. *J Clin Invest*, 110(11):1579–1583, Dec 2002. (Cited on pages 4 and 16)

[120] M. Phillips, R. N. Cataneo, B. A. Ditkoff, et al. Volatile markers of breast cancer in the breath. *Breast J*, 9(3):184–191, 2003. (Cited on pages 1 and 5)

[121] E. Piitulainen and S. Eriksson. Decline in FEV_1 related to smoking status in individuals with severe α_1-antitrypsin deficiency (PiZZ). *Eur Respir J*, 13(2):247–251, Feb 1999. (Cited on page 18)

[122] F. Podebrad, M. Heil, S. Reichert, et al. 4,5-Dimethyl-3-hydroxy-2[5*H*]-furanone (sotolone)—The odour of maple syrup urine disease. *J Inherit Metab Dis*, 22(2):107–114, Apr 1999. (Cited on page 5)

[123] W. A. Pryor and K. Stone. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Ann N Y Acad Sci*, 686:12–27, May 1993. (Cited on page 2)

[124] K. F. Rabe, S. Hurd, A. Anzueto, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med*, 176(6):532–555, Sep 2007. (Cited on pages 1 and 3)

[125] I. Rahman. Oxidative stress in pathogenesis of chronic obstructive pulmonary disease: cellular and molecular mechanisms. *Cell Biochem Biophys*, 43(1):167–188, Aug 2005. (Cited on page 2)

[126] R. Rocco, R. A. Incalzi, G. Pennazza, et al. Bionote e-nose technology may reduce false positives in lung cancer screening programmes. *Eur J Cardiothorac Surg*, Sep 2015. (Cited on page 19)

[127] T. Rogosch, N. Herrmann, R. F. Maier, et al. Detection of bloodstream infections and prediction of bronchopulmonary dysplasia in preterm neonates with an electronic nose. *J Pediatr*, 165(3):622–624, Sep 2014. (Cited on page 18)

[128] S. S. Salvi and P. J. Barnes. Chronic obstructive pulmonary disease in non-smokers. *Lancet*, 374(9691):733–743, Aug 2009. (Cited on page 1)

[129] L. H. Santo Tomas. Emphysema and chronic obstructive pulmonary disease in coal miners. *Curr Opin Pulm Med*, 17(2):123–125, Mar 2011. (Cited on page 1)

[130] S. T. Schmid, J. Koepke, M. Dresel, et al. The effects of weekly augmentation therapy in patients with PiZZ α1-antitrypsin deficiency. *Int J Chron Obstruct Pulmon Dis*, 7:687–696, Sep 2012. (Cited on page 17)

[131] Science Service. Science News. *Science*, 73(1892):10–14, Apr 1931. (Cited on page 5)

[132] A. M. Seiden. *Taste and Smell Disorders*. Rhinology and Sinusology. Thieme, 5th edition, 1997. (Cited on page 5)

[133] W. B. Shelley, H. J. Hurley, Jr., and A. C. Nichols. Axillary odor: experimental study of the role of bacteria, apocrine sweat, and deodorants. *AMA Arch Derm Syphilol*, 68(4):430–446, Oct 1953. (Cited on page 5)

[134] M. Shirasu and K. Touhara. The scent of disease: volatile organic compounds of the human body related to disease and disorder. *J Biochem*, 150(3):257–266, Sep 2011. (Cited on page 5)

[135] M. E. Shykhon, D. W. Morgan, R. Dutta, et al. Clinical evaluation of the electronic nose in the diagnosis of ear, nose and throat infection: a preliminary study. *J Laryngol Otol*, 118(9):706–709, Sep 2004. (Cited on page 6)

[136] S. Silbernagl and A. Despopoulos. *Taschenatlas Physiologie*. Thieme Electronic Book Library. Thieme, 7th edition, 2007. (Cited on page 5)

[137] Smiths Detection. *The Cyranose® 320 ENose user's manual*. 73 North, Vinedo Ave., Pasadena, CA 91107, revision F of 6th edition, Jan 2005. (Cited on page 15)

[138] H. Sonoda, S. Kohnoe, T. Yamazato, et al. Colorectal cancer screening with odour material by canine scent detection. *Gut*, 60(6):814–819, Jun 2011. (Cited on page 5)

[139] P. B. Sorroche, M. Fernández Acquier, O. López Jove, et al. Alpha-1 antitrypsin deficiency in copd patients: a cross-sectional study. *Arch Bronconeumol*, 51(11):539–543, Nov 2015. (Cited on page 3)

[140] J. K. Stoller, R. A. Sandhaus, G. Turino, et al. Delay in diagnosis of α_1-antitrypsin deficiency: a continuing problem. *Chest*, 128(4):1989–1994, Oct 2005. (Cited on pages 1 and 3)

[141] J. K. Stoller, P. Smith, P. Yang, and J. Spray. Physical and social impact of alpha 1-antitrypsin deficiency: results of a survey. *Cleve Clin J Med*, 61(6):461–467, 1994. (Cited on page 3)

[142] C. Strange, J. K. Stoller, R. A. Sandhaus, et al. Results of a survey of patients with alpha-1 antitrypsin deficiency. *Respiration*, 73(2):185–190, 2006. (Cited on page 3)

[143] M. J. Tobin, P. J. Cook, and D. C. Hutchison. Alpha_1 antitrypsin deficiency: the clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z. *Br J Dis Chest*, 77(1):14–27, Jan 1983. (Cited on page 3)

[144] A. R. Tonelli, F. Rouhani, N. Li, et al. Alpha-1-antitrypsin augmentation therapy in deficient individuals enrolled in the Alpha-1 Foundation DNA and Tissue Bank. *Int J Chron Obstruct Pulmon Dis*, 4:443–452, 2009. (Cited on page 5)

[145] J. Travis. Structure, function, and control of neutrophil proteinases. *Am J Med*, 84(6A):37–42, Jun 1988. (Cited on page 3)

[146] M. Troccaz, C. Starkenmann, Y. Niclass, et al. 3-Methyl-3-sulfanylhexan-1-ol as a major descriptor for the human axilla-sweat odour profile. *Chem Biodivers*, 1(7):1022–1035, Jul 2004. (Cited on page 5)

[147] L. Trupin, G. Earnest, M. San Pedro, et al. The occupational burden of chronic obstructive pulmonary disease. *Eur Respir J*, 22(3):462–469, Sep 2003. (Cited on page 1)

[148] C. Vogelmeier and H. Worth. [COPD - a historical review, current management and research perspectives]. *Pneumologie*, 64(9):550–554, Sep 2010 (German). (Cited on page 2)

[149] K. von Frisch. Decoding the language of the bee. *Science*, 185(4152):663–668, Aug 1974. (Cited on page 5)

[150] T. Vos, A. D. Flaxman, M. Naghavi, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 380(9859):2163–2196, Dec 2012. (Cited on page 1)

[151] R. H. Waring, S. C. Mitchell, and G. R. Fenwick. The chemical nature of the urinary odour produced by man after asparagus ingestion. *Xenobiotica*, 17(11):1363–1371, Nov 1987. (Cited on page 5)

[152] M. Wencker, B. Fuhrmann, N. Banik, and N. Konietzko. Longitudinal follow-up of patients with α_1-protease inhibitor deficiency before and during therapy with IV α_1-protease inhibitor. *Chest*, 119(3):737–744, Mar 2001. (Cited on page 5)

[153] M. Westhoff, P. Litterst, L. Freitag, et al. Ion mobility spectrometry for the detection of volatile organic compounds in exhaled breath of patients with lung cancer: results of a pilot study. *Thorax*, 64(9):744–748, Sep 2009. (Cited on page 7)

[154] M. Westhoff, P. Litterst, S. Maddula, et al. Statistical and bioinformatical methods to differentiate chronic obstructive pulmonary disease (COPD) including lung cancer from healthy control by breath analysis using ion mobility spectrometry. *Int J Ion Mob Spec*, 14(4):139–149, Dec 2011. (Cited on page 6)

[155] M. Westhoff, P. Litterst, S. Maddula, et al. Differentiation of chronic obstructive pulmonary disease (COPD) including lung cancer from healthy control group by breath analysis using ion mobility spectrometry. *Int J Ion Mobil Spec*, 13(3–4):131–139, Dec 2010. (Cited on page 7)

[156] H. R. W. Wirtz and M. Schmidt. Acute influence of cigarette smoke on secretion of pulmonary surfactant in rat alveolar type II cells in culture. *Eur Respir J*, 9(1):24–32, Jan 1996. (Cited on page 2)

[157] I. S. Woolhouse, D. L. Bayley, and R. A. Stockley. Sputum chemotactic activity in chronic obstructive pulmonary disease: effect of α_1-antitrypsin deficiency and the role of leukotriene B_4 and interleukin 8. *Thorax*, 57(8):709–714, Aug 2002. (Cited on page 16)

[158] J. Yan, X. Guo, S. Duan, et al. Electronic nose feature extraction methods: a review. *Sensors (Basel)*, 15(11):27804–27831, Nov 2015. (Cited on page 17)

[159] T. Zakharkina, A.-R. Koczulla, O. Mardanova, et al. Detection of microorganisms in exhaled breath condensate during acute exacerbations of COPD. *Respirology*, 16(6):932–938, Aug 2011. (Cited on page 6)

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ORIGINAL ARTICLE

Discrimination between COPD patients with and without alpha 1-antitrypsin deficiency using an electronic nose

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ABSTRACT

Background and objective: **To compare the volatile organic compound patterns of patients with COPD with and without alpha 1-antitrypsin (AAT) deficiency using electronic nose technology.**

Methods: **Exhaled breath condensate and pure exhaled breath of patients with COPD with ($n = 10$) and without ($n = 23$) AAT deficiency and healthy controls ($n = 10$) were analysed. The effect of human recombinant AAT on the volatile organic compound profile of 11 AAT-deficient patients was also examined. Exhaled breath condensate and pure exhaled breath were measured using the Cyranose 320. Smell prints were analysed by linear discriminant analysis (LDA) using Mahalanobis distance (MD) and cross-validation values (CVVs).**

Results: **Smell prints of patients with AAT-deficiency were different from those with COPD in exhaled breath condensate (LDA: $P < 0.0001$, sensitivity of 1.00, specificity of 1.00, CVV 82.0%, MD 2.37) and in pure exhaled breath (LDA: $P < 0.0001$, sensitivity of 1.00, specificity of 1.00, CVV 58.3%, MD 2.27). Smell prints of AAT-deficient patients before and after human recombinant AAT augmentation were different (LDA: $P = 0.001$, sensitivity of 1.00, specificity of 1.00, CVV 53.3%, MD 1.79).**

Conclusions: **An electronic nose can detect differences in smell prints of COPD patients with and without AAT deficiency. Augmentation therapy changes the volatile organic compound pattern. The electronic nose may be helpful in the diagnosis of AAT deficiency.**

SUMMARY AT A GLANCE

This study demonstrates that an electronic nose can distinguish the exhaled breath of patients with chronic obstructive pulmonary disease with and without AAT deficiency. Furthermore, it can detect an acute effect of human recombinant AAT on exhaled breath.

Key words: alpha 1-antitrypsin deficiency, chronic obstructive pulmonary disease, electronic nose, exhaled breath, volatile organic compound.

INTRODUCTION

In current clinical practice, COPD is diagnosed and monitored via symptoms, lung function and the assessment of responses to inhaled pharmacological agents. These tests have been standardized and are generally considered as informative.[1] However, the information provided by these tests is still limited, and some of them are complex or time-consuming, which has limited their implementation in medical care.

Alpha 1-antitrypsin (AAT) deficiency is a co-dominant inherited disorder that is diagnosed by genotyping and low serum levels of AAT. The costs of diagnostic procedures are high;[2] there is therefore a need for novel diagnostic methods that are simple, fast and cost-effective. Within the past decade, cellular and molecular techniques have been utilized as options for the diagnosis and monitoring of COPD and AAT deficiency.[3]

The analysis of exhaled breath (EB) has been used to non-invasively obtain information about inflammatory processes within the lung. EB contains a complex mixture of volatile organic compounds (VOCs), which can be detected using gas chromatography-mass spectrometry (GC-MS).[4] Electronic noses (eNoses) use a concept essentially different from GC-MS. They allow online recognition of complex VOC mixtures via

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Table 1 Patient characteristics

	AATD		COPD		Healthy controls	
	EBC	PEB	EBC	PEB	EBC	PEB
Mean age in years (standard deviation)	55 (8)	58 (8)	66 (6)	63 (8)	30 (7)	51 (7)
Number ♀/♂	2/8	3/7	2/8	5/18	6/4	7/3
Mean % FEV_1 (standard deviation)	47.9 (16.6)	41.8 (18.5)	53.1 (13.2)	56.5 (19.5)	105.2 (11.1)	N/A
Smoker current/ex	0/9	0/7	1†/9	2/21	0/2	0/2

† Pipe.
AATD, alpha 1-antitrypsin deficiency; EBC, exhaled breath condensate; N/A, not available; PEB, pure exhaled breath.

composite nanosensor arrays in combination with learning algorithms.[5,6] Each sensor reacts differently to a certain VOC mixture. The arrays combine discrimination performance with sensitivity, short response time and reversible behaviour.[5] Data analysis follows a heuristic approach, allowing the distinction of 'smell prints' from various sources by pattern-recognition algorithms, thus providing discrimination between gas mixtures without the identification of (all) individual molecular components. The first proof-of-concept studies in respiratory medicine demonstrated high accuracy in the *ex vivo* classification of bacterial infections and promising discrimination of EB obtained from patients with lung cancer and controls.[7] Furthermore, inflammatory diseases, for example, asthma and COPD, could be discriminated.[3,7]

AAT deficiency is known to predispose to COPD and is symptomatically treated as COPD.[1] It is currently assumed that COPD with or without AAT deficiency shows different molecular and cellular characteristics due to the specific deficiency present in AAT deficiency.[8] Thus it may be that the VOC profile is different in the EB of COPD patients with or without AAT deficiency. Based on this possibility, it was the aim of our study to compare smell prints between COPD patients with confirmed AAT deficiency, COPD patients without AAT deficiency and healthy controls. eNose technology was used to directly analyze EB, and in addition, the composition of conventionally obtained exhaled breath condensate (EBC) was analysed to gain an understanding of the VOC patterns and to differentiate between the diseases.

METHODS

Two experimental protocols were used. In the first, we collected EBC samples and analyzed them by eNose; in the second, the pure exhaled breath (PEB) was collected in a sample bag and these samples were examined by eNose. In the EBC analysis, we included 10 healthy control subjects, 10 patients with proven AAT deficiency and 10 patients with COPD in order to check for hints into the direction of the hypothesis in a proof-of-concept study. Afterwards, for the PEB experiment, we again recruited 10 healthy control subjects, 10 AAT-deficient patients and 23 COPD patients. Unfortunately, not all subjects were available for the second approach, thus the groups were only identical in part.

Study participants

In the first experiment we included patients with stable COPD (GOLD* stages II and III), with no signs of exacerbation (Table 1). No patient had received treatment with oral steroids for at least 4 weeks before the measurements. Confirmation of AAT deficiency was done by PCR and isoelectric focusing. Patients with AAT deficiency received a weekly weight-adapted AAT augmentation therapy; this was left unchanged during the course of the study. In patients who had COPD without AAT deficiency, low AAT levels were ruled out by serum analysis. The healthy control subjects did not show signs of pulmonary disorders from lung function or clinical history. The patients and subjects who participated in the second experiment were characterized by analogous procedures.

All participants answered a questionnaire regarding symptoms, smoking habits, health status, medication and medical history (Table 1). The study was approved by the local ethics committee (Marburg Ethics Committee 59/06) and informed consent was obtained from each subject.

eNose

For both experiments the Cyranose 320 (C-320) (Smiths Detection Group Ltd, Watford, UK) was used. This is a hand-held device capable of detecting so-called smell prints by analyzing mixtures of VOCs. The C-320 is equipped with 32 chemical sensors that respond differently to different VOC mixtures. The sensors consist of conducting chemiresistors made from carbon black nanocomposites that change their resistance in response to VOCs.

One measurement with the C-320 included three consecutive steps:
1 Baseline: The sensors were exposed to reference air.

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2 Sampling: The sensors were exposed to sample air. The changes of sensor resistances compared with reference air were recorded.
3 Purging: The sensors were refreshed by exposing them to ambient air.

Measurements

EBC

EBC samples were collected during 10 min of tidal breathing through a single-use disposable RTube device (Respiratory Research Inc., Austin, TX, USA). A nose clip was applied to each subject during EBC sampling. Before sampling, the collecting tube was cooled in a refrigerator to a temperature of –20°C. Immediately after collection, EBC was transferred into polyethylene tubes (Eppendorf AG, Hamburg, Germany) that had been washed with double-deionized water. The collected samples were stored in reaction tubes at –20°C. Samples were deaerated with argon (99.9% purity, Linde Gases, Munich, Germany) and frozen at –80°C.

For the measurement with the eNose, 200 μL of EBC was warmed to 37°C and gently bubbled with argon gas for 2 min to facilitate evaporation. Ambient air was used as baseline for 10 s. The snout of the C-320 was held above the surface drawing a sample for 10 s.

PEB

The participants breathed medicinal air (Aer medicinalis Linde, Linde Gas Therapeutics GmbH, Unterschleißheim, Germany) and exhaled for 10 s at a flow rate of 100–200 mL/s into a collection bag (Fig. 1). This medicinal air was also used as reference air for the 60-s baseline, followed by a 60-s sample draw from the collection bag. These measurements were performed in triplicate.

In patients with AAT deficiency, additional PEB measurements were performed 6 days after augmentation therapy. Human purified AAT (Prolastin, Talecris Biotherapeutics, Frankfurt, Germany) was used for augmentation therapy in a dose of 60 mg/kg body weight.

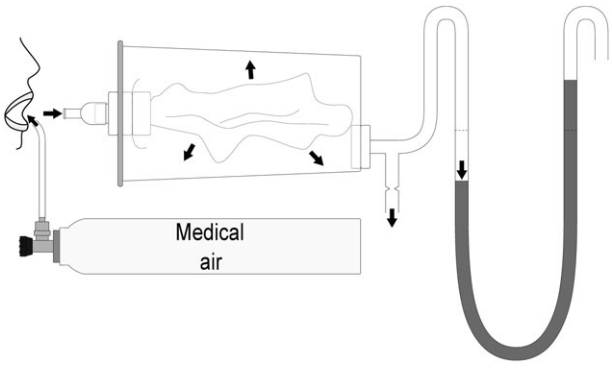

Figure 1 Schematic design of the pure exhaled breath sampling. Including bag-in-box system, medical air and water column for constant expiratory flow.

Data analysis

Linear discriminant analysis (LDA) was used to distinguish between groups. A LDA estimates a linear function that discriminates two groups and represents the data in a one-dimensional score. In order to improve comparability, for the PEB experiment repeated measurements from each participant were averaged by computing arithmetic means and the results normalised to a range from 0 to 1. For the PEB before and after therapy experiment, the data was averaged only. For the EBC experiment the data was normalised only as no repeated measurements were done.

As a variance-dependent distance measure for multidimensional data the Mahalanobis distance (MD)[a] between groups was used. A MD greater than 1.96 was considered significant as this exceeds a 95% confidence level, corresponding to a *P*-value smaller than 0.05. A MD exceeding 2.58 even satisfies a *P*-value smaller 0.01 and corresponds to a confidence level of 99%. The MD was favored over the 'usual' Euclidean distance because the latter does not take into account the variability of the data.

A *k*-fold cross-validation was performed in each run in order to calculate the cross-validation value (CVV). We used a leave-one-out procedure, in which one data sample of each group was left out, and $k = n_1 \cdot n_2$, with n_1 and n_2 being the sample sizes of groups 1 and 2. The CVV yielded the proportion of correct predictions in a reduced data set in the EBC and PEB experiment, as well as for the augmentation data. The values of the LDA were used in a Mann–Whitney *U*-test and a Wilcoxon signed-rank test for paired groups (augmentation data), respectively.

RESULTS

Patient characteristics can be found in Table 1.

Analysis of EBC by eNose

The LDA is able to separate the three groups as shown in the canonical plot (Fig. 2). Each of the groups—AAT deficiency, COPD and healthy controls—forms a separate cluster and is clearly distinguishable. The LDA provides excellent performance and statistically significant differences ($P < 0.0001$) in the distinction between groups. Also the CVV performs well with 82.0% between AAT deficiency and COPD ($P < 0.0001$, sensitivity of 1.00, specificity of 1.00, these three parameters refer to the LDA values), 80.5% between healthy controls and COPD ($P < 0.0001$, sensitivity of 1.00, specificity of 1.00), and 59.5% comparing

[a]$MD(x, y) = ((x - y)^T C^{-1}(x - y))^{1/2}$, with $x = (x_1, \ldots, x_n)$, $y = (y_1, \ldots, y_n)$ and C^{-1} as the inverse covariance matrix of the *n*-dimensional data.

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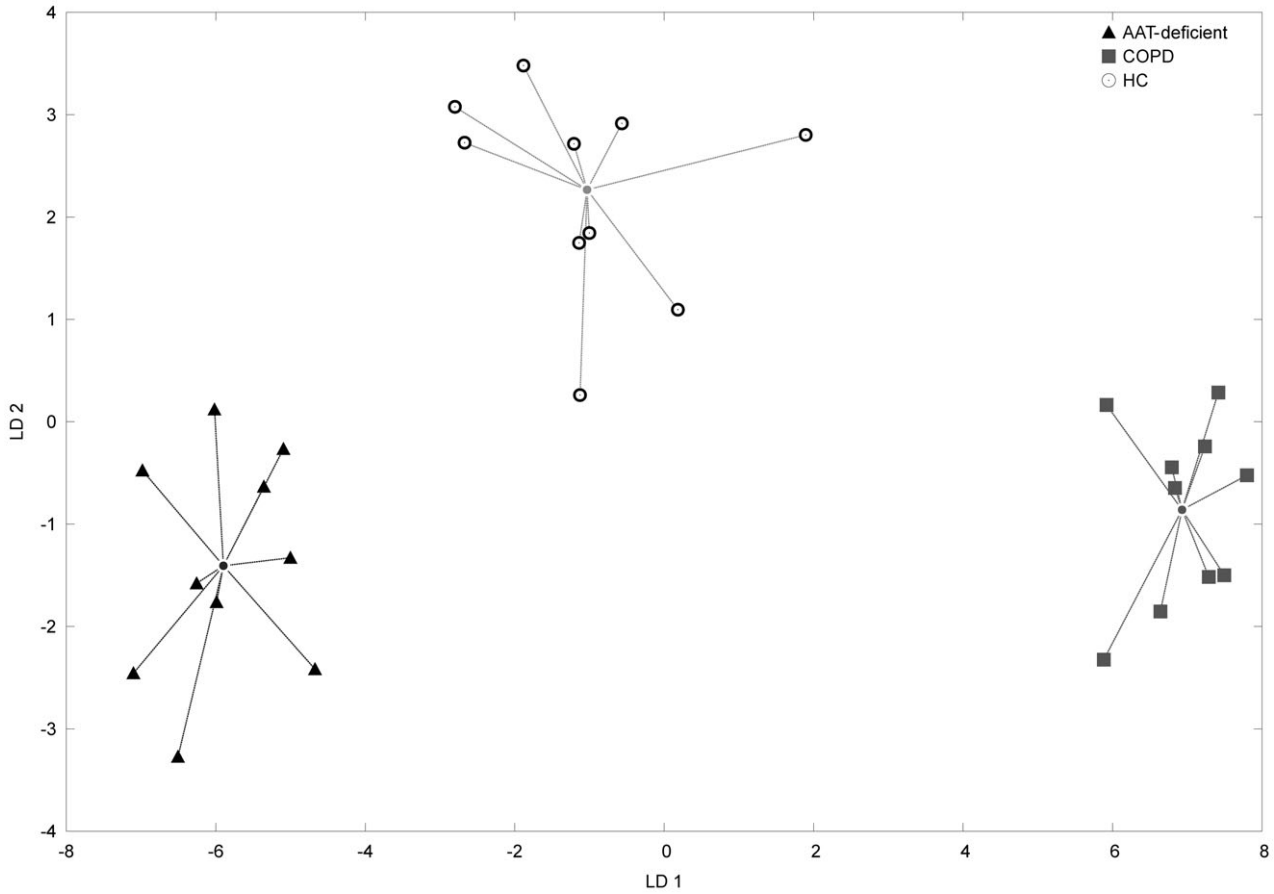

Figure 2 Canonical plot of Cyranose 320 results from exhaled breath condensate analysis of the three groups. Alpha 1-antitrypsin (AAT)-deficient (▲) and COPD (■) patients and healthy controls (HC) (○) form separate clusters. Cross-validation indicated good values of discrimination (59.5–82.0%). LD: Value of linear discriminant.

healthy controls with AAT deficiency ($P < 0.0001$, sensitivity of 1.00, specificity of 1.00). The MDs indicates that the distance between AAT deficiency and COPD is slightly higher than the other comparisons. Comparing the group of AAT deficiency patients with COPD patients, the distance is 2.37. Comparing AAT deficiency with healthy controls, the distance is 2.12. COPD and healthy controls shows a Mahalanobis distance of 2.19.

Analysis of PEB by eNose

Each of the groups, AAT deficiency, COPD and healthy controls, forms a separate cluster and is clearly distinguishable in a three-dimensional plot (Fig. 3).

The LDA provides statistically significant differences for all comparisons ($P < 0.0001$). The CVV between AAT deficiency and COPD is 58.3% ($P < 0.0001$, sensitivity of 1.00, specificity of 1.00, these three parameters refer to the LDA values). The CVV between AAT-deficient patients and healthy controls is 62.0% ($P < 0.0001$, sensitivity of 1.00, specificity of 1.00) and 67.6% comparing COPD with healthy controls ($P < 0.0001$, sensitivity of 1.00, specificity of 1.00).

MDs in the PEB experiment (2.27–2.67) are slightly higher than in the EBC experiment (2.12–2.37).The MD between AAT deficiency and COPD is 2.27. Comparing AAT deficiency patients with healthy controls, the distance is 2.67. When comparing COPD with healthy controls, the distance is 2.28.

Analysis of PEB before and after therapy

EB of 11 AAT deficiency patients was analysed 6 days after augmentation and 2–3 h after infusion of the augmentation therapy with human recombinant AAT. The MD is 1.79, showing a CVV of 53.3% (Fig. 4, $P = 0.001$, sensitivity of 1.00, specificity of 1.00, these three parameters refer to the LDA values).

DISCUSSION

Our study indicates that an eNose can distinguish the pattern of VOCs present in the EBC of patients with AAT deficiency from those with COPD. Moreover, it could also distinguish between the PEB of AAT deficiency and COPD patients. The eNose also

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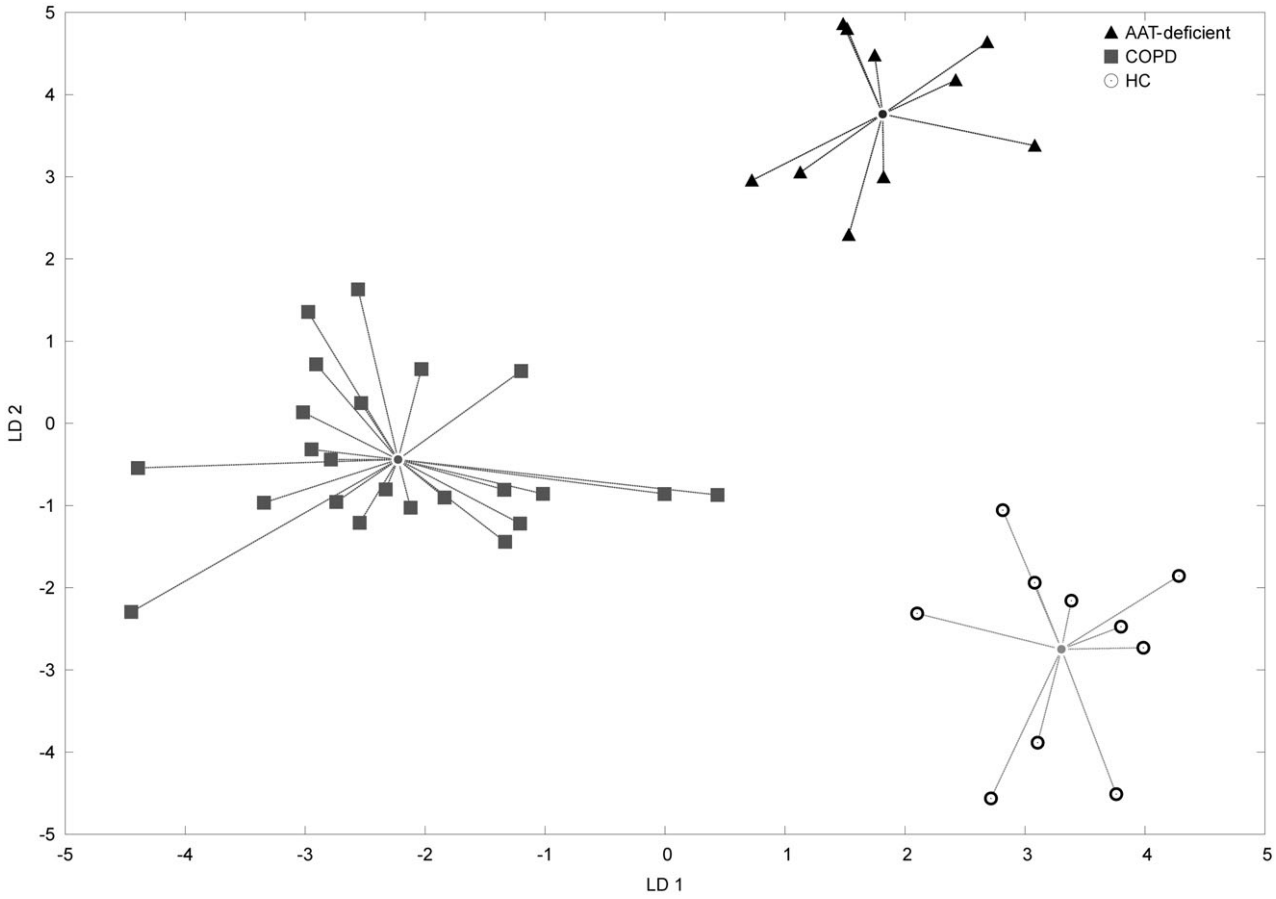

Figure 3 Canonical plot of Cyranose 320 results from pure exhaled breath analysis of the three groups. Alpha 1-antitrypsin (AAT)-deficient (▲) and COPD (■) patients and healthy controls (HC) (○) form separate clusters. Cross-validation was in the range (58.3 to 67.6%). LD: Value of linear discriminant.

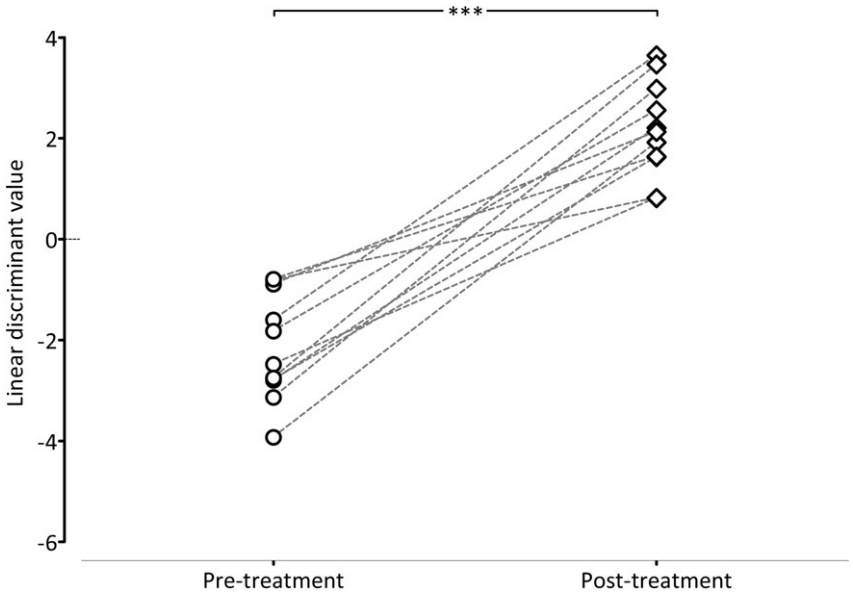

Figure 4 Linear discriminant analysis (LDA) of the exhaled breath condensate data of augmented alpha 1-antitrypsin deficiency patients before (pre-treatment) and after augmentation therapy (post-treatment). The LDA distinguished between the augmented and non-augmented alpha 1-antitrypsin deficiency patients (***$P = 0.001$).

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discriminated between patients with either disease from control subjects. It was even possible to distinguish between AAT deficiency patients before and after human recombinant AAT therapy. This suggests that the VOC patterns differ between the two lung diseases, although they share common clinical features. Furthermore, the augmentation with AAT appears to lead to a change in VOC profile.

EB analysis has been widely used to obtain information about inflammatory diseases of the lung. EB contains hundreds of VOCs, as established using GC-MS.[4] eNoses are a novel approach of VOC detection via composite nanosensor arrays,[5] but in most cases they do not allow the identification of individual molecular components.[6] In addition to sensor-based equipment, more recently introduced devices such as ion mobility spectrometers also provide promising opportunities for the analysis of VOCs.[9]

We aimed to determine whether patients with AAT deficiency could be discriminated by smell prints from patients with COPD and non-smoking healthy controls, using either direct analysis of PEB or indirect analysis by measuring the headspace above EBC samples. The relatively high CVVs in both experiments suggest that the LDA works well on these kinds of samples. The smaller CVVs of the PEB analysis could be attributed to the fact that these samples had a larger variance than the EBC samples. To our knowledge, this is the first study to compare the EBC smell prints of COPD with AAT deficiency with those of COPD only.

The first studies of eNoses in the field of respiratory medicine indicated high accuracy in the *ex vivo* classification of bacterial infection.[10,11] Preliminary clinical studies used an eNose for the diagnosis of ear, nose and throat infections[12] as well as for the diagnosis of pneumonia.[13] Furthermore, EB from patients with lung cancer exhibited smell prints different from those of controls[14] or patients with a variety of other lung diseases,[15] including COPD.[7]

With regard to asthma and COPD, a number of studies with inconsistent results have been published. Smell prints of asthma patients could be discriminated from those of healthy controls[16] or non-smoking COPD patients.[3] On the other hand, no significant difference between COPD (or AAT deficiency) patients and non-smoking healthy controls was found.[3,14] In these studies, it was speculated that the lack of significant difference might have been due to a lack of active inflammation in patients with COPD. However, in general, the presence of airway inflammation, both acute and chronic, is well documented in COPD patients.[17] Another study has shown that the subjects' age did not have a significant impact on VOCs detectable by the eNose.[3] In order to rule out that differences in FEV_1 between AAT-deficient and COPD patients did affect our conclusions, multiple logistic regression analyses were performed. For both approaches, EBC and PEB, no significant relationship was found.

Our results provide evidence that patients with COPD, severe AAT deficiency (PiZ) and non-smoking healthy controls exhibit different smell prints, possibly due to different types of (systemic) inflammation. This is supported by studies that have reported differences in airway inflammation between AAT deficiency and COPD. Both a higher level[18] and a lower level of fraction of exhaled nitric oxide in AAT-deficient (PiMZ) subjects compared with COPD patients have been found.[19] Neutrophil chemotactic activity in sputum appears to be significantly higher in AAT deficiency (PiZ) than in COPD.[20] It is very likely that the specific defect in AAT deficiency has a multitude of cellular and biochemical consequences, including the more systemic aspect.

EB comprises a gaseous phase containing VOCs (such as NO and CO) and a liquid phase, also called EBC.[21] EBC contains aerosol particles, in which a number of non-volatile molecules have been identified.[22–26] The examination of EBC might be considered as a non-invasive method for studying the composition of airway lining fluid.[25–28] About 4% of the EBC in healthy subjects is derived from aerosolized airway lining fluid.[29] Our data suggest that EBC also contains compounds that are volatile during degassing, and which can therefore be recognized by the eNose.

A major difference in comparing the PEB and the EBC was that an additional step had to be performed in the latter approach. On the other hand, EBC is storable and can be analysed at a later time, while the PEB is not storable, as VOCs might get lost. It is unclear whether the condensation of exhaled air affects the detection of compounds, and this has to be considered with regard to targets and methods chosen for analysis.

From the data on AAT deficiency patients before and after treatment with purified human AAT, it can be speculated that AAT leads to a change in VOC profile. It has been demonstrated that AAT is directly measurable in the bronchoalveolar lavage fluid after this treatment;[30] it is therefore likely that the treatment with AAT leads to changes in VOC both locally and systemically. An inflammatory response after augmentation therapy cannot be excluded because of the administration of pooled human proteins.

Using a serial test approach for the diagnosis of AAT deficiency comprising measurement of the serum level of AAT, isoelectric focusing, and/or PCR for Z and S alleles, the majority of patients with AAT deficiency can be identified, although there is still an occasional need for genome sequence analysis.[2] However, this diagnostic algorithm is time-consuming and expensive, and novel procedures are desirable. They should be simple, accurate, fast and cost-effective. The use of the eNose in the diagnosis of AAT deficiency should be evaluated further.

There are important limitations to this study. First, we investigated a small number of patients and the training set used here requires validation in a completely separate cohort. This is in line with the STARD (standards for the reporting of diagnostic accuracy studies) statement for the validation of diagnostic tests.[31]

In conclusion the eNose Cyranose 320 can detect differences in smell prints in COPD with or without AAT deficiency. AAT therapy changes the VOC pattern. It suggests that an eNose might be helpful in the

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assessment of AAT deficiency. Both methods, eNose and EBC, can be used in larger trials as they are non-invasive and sampling is simple to perform.

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REFERENCES

1 Rabe KF, Hurd S, Anzueto A *et al*. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am. J. Respir. Crit. Care Med.* 2007; **176**: 532–55.
2 Bals R, Koczulla R, Kotke V *et al*. Identification of individuals with alpha-1-antitrypsin deficiency by a targeted screening program. *Respir. Med.* 2007; **101**: 1708–14.
3 Fens N, Zwinderman AH, van der Schee MP *et al*. Exhaled breath profiling enables discrimination of chronic obstructive pulmonary disease and asthma. *Am. J. Respir. Crit. Care Med.* 2009; **180**: 1076–82.
4 Moser B, Bodrogi F, Eibl G *et al*. Mass spectrometric profile of exhaled breath–field study by PTR-MS. *Respir. Physiol. Neurobiol.* 2005; **145**: 295–300.
5 Lewis NS. Comparisons between mammalian and artificial olfaction based on arrays of carbon black-polymer composite vapor detectors. *Acc. Chem. Res.* 2004; **37**: 663–72.
6 Thaler ER, Hanson CW. Medical applications of electronic nose technology. *Expert Rev. Med. Devices* 2005; **2**: 559–66.
7 Dragonieri S, Annema JT, Schot R *et al*. An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. *Lung Cancer* 2009; **64**: 166–70.
8 Holme J, Stockley RA. Radiologic and clinical features of COPD patients with discordant pulmonary physiology: lessons from alpha1-antitrypsin deficiency. *Chest* 2007; **132**: 909–15.
9 Baumbach JI. Ion mobility spectrometry coupled with multicapillary columns for metabolic profiling of human breath. *J. Breath. Res.* 2009; **3**: 034001.
10 Lai SY, Deffenderfer OF, Hanson W *et al*. Identification of upper respiratory bacterial pathogens with the electronic nose. *Laryngoscope* 2002; **112**: 975–9.
11 Dutta R, Hines EL, Gardner JW *et al*. Bacteria classification using Cyranose 320 electronic nose. *Biomed. Eng. Online* 2002; **1**: 4.
12 Shykhon ME, Morgan DW, Dutta R *et al*. Clinical evaluation of the electronic nose in the diagnosis of ear, nose and throat infection: a preliminary study. *J. Laryngol. Otol.* 2004; **118**: 706–9.
13 Hockstein NG, Thaler ER, Torigian D *et al*. Diagnosis of pneumonia with an electronic nose: correlation of vapor signature with chest computed tomography scan findings. *Laryngoscope* 2004; **114**: 1701–5.
14 Machado RF, Laskowski D, Deffenderfer O *et al*. Detection of lung cancer by sensor array analyses of exhaled breath. *Am. J. Respir. Crit. Care Med.* 2005; **171**: 1286–91.
15 D'Amico A, Pennazza G, Santonico M *et al*. An investigation on electronic nose diagnosis of lung cancer. *Lung Cancer* 2010; **68**: 170–6.
16 Dragonieri S, Schot R, Mertens BJ *et al*. An electronic nose in the discrimination of patients with asthma and controls. *J. Allergy Clin. Immunol.* 2007; **120**: 856–62.
17 Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. *J. Clin. Invest.* 2008; **118**: 3546–56.
18 Malerba M, Ragnoli B, Radaeli A. Exhaled nitric oxide levels in alpha-1-antitrypsin PiMZ subjects. *J. Intern. Med.* 2009; **265**: 382–7.
19 Machado RF, Stoller JK, Laskowski D *et al*. Low levels of nitric oxide and carbon monoxide in alpha 1-antitrypsin deficiency. *J. Appl. Physiol.* 2002; **93**: 2038–43.
20 Woolhouse IS, Bayley DL, Stockley RA. Sputum chemotactic activity in chronic obstructive pulmonary disease: effect of alpha(1)-antitrypsin deficiency and the role of leukotriene B(4) and interleukin 8. *Thorax* 2002; **57**: 709–14.
21 Montuschi P. Analysis of exhaled breath condensate in respiratory medicine: methodological aspects and potential clinical applications. *Ther. Adv. Respir. Dis.* 2007; **1**: 5–23.
22 Carpagnano GE, Kharitonov SA, Resta O *et al*. Increased 8-isoprostane and interleukin-6 in breath condensate of obstructive sleep apnea patients. *Chest* 2002; **122**: 1162–7.
23 Hoffmeyer F, Raulf-Heimsoth M, Bruning T. Exhaled breath condensate and airway inflammation. *Curr. Opin. Allergy Clin. Immunol.* 2009; **9**: 16–22.
24 Kharitonov SA, Barnes PJ. Exhaled markers of inflammation. *Curr. Opin. Allergy Clin. Immunol.* 2001; **1**: 217–24.
25 Montuschi P, Barnes PJ. Exhaled leukotrienes and prostaglandins in asthma. *J. Allergy Clin. Immunol.* 2002; **109**: 615–20.
26 Montuschi P, Martello S, Felli M *et al*. Liquid chromatography/mass spectrometry analysis of exhaled leukotriene B4 in asthmatic children. *Respir. Res.* 2005; **6**: 119.
27 Effros RM, Biller J, Foss B *et al*. A simple method for estimating respiratory solute dilution in exhaled breath condensates. *Am. J. Respir. Crit. Care Med.* 2003; **168**: 1500–5.
28 Montuschi P, Nightingale JA, Kharitonov SA *et al*. Ozone-induced increase in exhaled 8-isoprostane in healthy subjects is resistant to inhaled budesonide. *Free Radic. Biol. Med.* 2002; **33**: 1403–8.
29 Dwyer TM. Sampling airway surface liquid: non-volatiles in the exhaled breath condensate. *Lung* 2004; **182**: 241–50.
30 Hubbard RC, McElvaney NG, Sellers SE *et al*. A-produced alpha 1-antitrypsin administered by aerosol augments lower respiratory tract antineutrophil elastase defenses in individuals with alpha 1-antitrypsin deficiency. *J. Clin. Invest.* 1989; **84**: 1349–54.
31 Bossuyt PM, Reitsma JB. The STARD initiative. *Lancet* 2003;**361**: 71.

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Contribution

My personal contribution to the aforementioned publication [59]:

- measuring of all participants and samples via EN
- most of the data acquisition
- entire data analysis
- large parts of the methods, results, and discussion chapters
- all tables and figures

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TECHNICAL REPORT

MCC/IMS as potential noninvasive technique in the diagnosis of patients with COPD with and without alpha 1-antitrypsin deficiency

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Background

Chronic obstructive lung disease (COPD) is characterized by a not fully reversible and usually progressive airflow limitation. The disease is associated with an inflammatory response of the lungs to noxious particles, mainly cigarette smoke but also alpha 1-antitrypsin (AAT) deficiency predisposes to COPD. The usual clinical practice for diagnosing COPD is following symptoms, performing lung function and the assessment of responses to inhaled pharmacological agents. These tests have been standardized and are generally considered as informative [1]. The tests are time consuming. Still the quality of the tests is influenced by experience and may differ depending where the tests are performed. Further, it is suggested that every COPD patient is once screened for AAT deficiency (AATD). Alpha 1-antitrypsin deficiency is a co-dominant inherited disorder that is diagnosed by low serum levels, genotyping and phenotyping of AAT.

Usually the serum AAT levels are analyzed first. If the serum levels are decreased further procedures like genotyping (with polymerase chain reaction) and phenotyping (isoelectric focusing) are suggested. The costs of these diagnostic procedures are high [2] and therefore often not carried out. Thus, there is a need for novel diagnostic methods that are simple, fast and cost-effective and maybe performed bedside. Within the past decade, cellular and molecular techniques have been utilized as options for the diagnosis and monitoring of COPD and AAT deficiency [3].

The analysis of exhaled breath (EB) has been used to noninvasively obtain information about inflammatory processes within the lung. EB contains a complex mixture of volatile organic compounds (VOCs), which can be detected using gas chromatography–mass spectrometry (GC-MS) [4]. Electronic noses (eNoses) use a concept essentially different from GC-MS. In addition, they allow the online recognition of complex VOC mixtures via composite nanosensor arrays in combination with learning algorithms [5, 6]. Another approach is the ion mobility spectroscopy (IMS), were about 10 ml human breath will be analyzed directly and without any pre-enrichment. Two different types of IMS, such coupled to multi-capillary columns (MCC/IMS) [7–15] and differential mobility spectrometers [16, 17] were used.

It is currently assumed that COPD with or without AAT deficiency shows different molecular and cellular characteristics due to the pathophysiological inflammation present in AAT deficiency [18]. Thus, it may be that the VOC profile or smellprint is different in the EB of patients with COPD

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Table 1 Characteristics of ion mobility spectrometer (BioScout 2010)

Parameter	Value
Ionization source	^{63}Ni (555 MBq)
Electric field strength	320 V/cm
Length of drift region	12 cm
Diameter of drift region	15 mm
Length of ionization chamber	15 mm
Shutter opening time	300 µs
Shutter impulse time	100 ms
Drift gas	synthetic air (20.5 % O_2 (4.5), 79.5 % N_2 (5.0))
Drift gas flow	100 ml/min to 300 ml/min
Pressure	101 kPa (ambient pressure)
Multi-capillary column	OV-5, polar
Column temperature	40 °C

with and without AAT deficiency. Based on this possibility, it was the aim of our study to compare smellprints between COPD patients with confirmed AAT deficiency and COPD patients without AAT deficiency. In another approach also the influence of AAT augmentation therapy should be studied. Patients with severe AATD can be treated with weekly AAT infusions (60 mg/kg body weight). This therapy regimen is based on studies showing an increase of AAT lung levels as well as an increase of the antineutrophil elastase capacity in the epithelial lining fluid of the lung [1]. Considering this, an influence of the augmentation on the smellprint of AATD patients seems

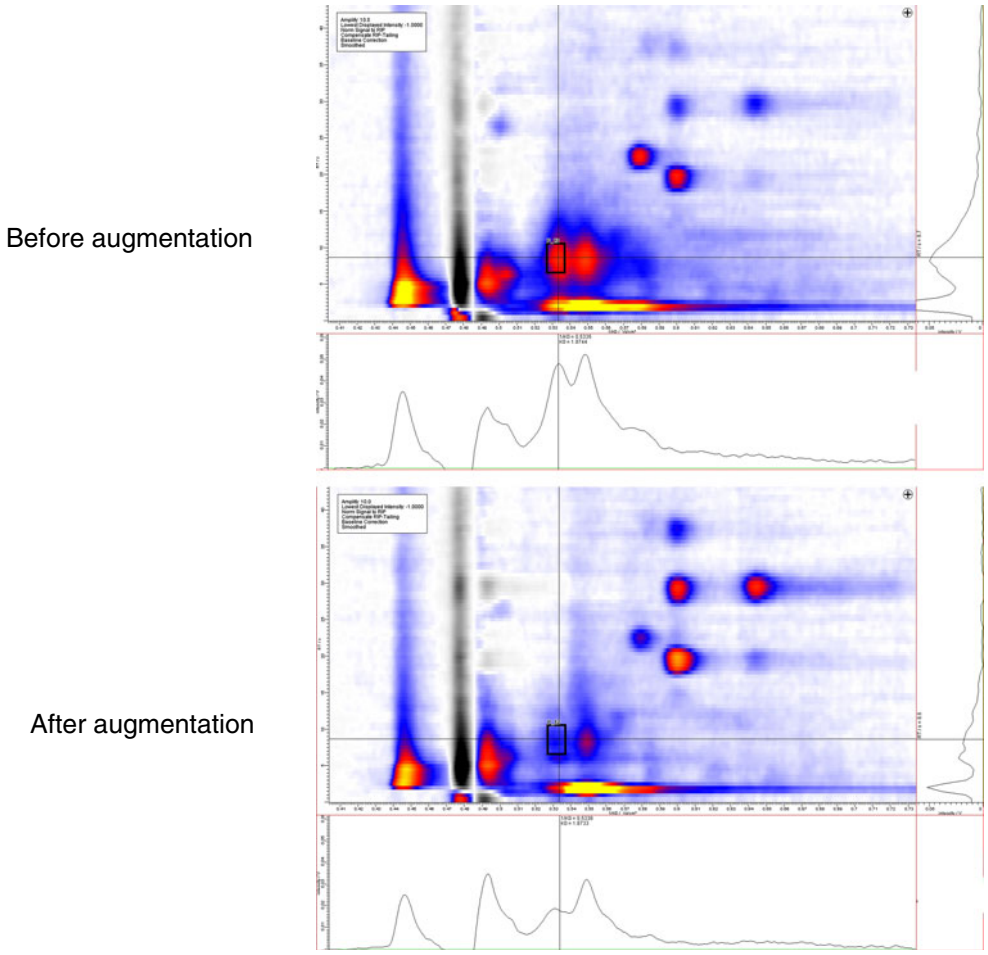


Fig. 1 IMS chromatograms before and after AATD augmentation

reasonable. Hence, the second aim of the study was to detect the influence of intravenously given AAT on the composition of VOCs in EB of AATD patients.

Patients

We included patients with genetically proved severe AATD (PiZZ genotype). All patients were fasting for at least 2 h. Patients had to wash out their mouth with water. For sampling the patients breathed into the device. For every breath the first expiratory 40 ml were discarded to exclude air of the oral cavity. The patients breathed until 10 ml of expiratory air (excluding the oral cavity) were obtained by the device for sampling. From AATD patients breath samples were taken twice, directly before and 2 h past their infusion or augmentation. The numbers of breath samples included within the two different cases investigated are:

- Before and after substitution: 2
- COPD without AATD: 8
- COPD based on AATD: 17.

Method

The IMS coupled to a multi-capillary column (MCC/IMS) used was a BioScout (B&S Analytik, Dortmund, Germany) consisting of the MCC/IMS and a SpiroScout (Ganhorn Medizin Electronic, Niederlauer, Germany) as sample inlet unit. The major parameters are summarized elsewhere [8–12, 19–23]. In this spectrometer a 550 MBq ^{63}Ni β-radiation source was applied for the ionization of the carrier gas (air). It was connected to a polar multi-capillary column (MCC, type OV-5, Multichrom Ltd, Novosibirsk, Russia) used as the pre-separation unit. In this MCC the analytes of exhaled breath were sent through 1000 parallel capillaries, each with an inner diameter of 40 µm and a film thickness of 200 nm. The total diameter of the separation column was 3 mm. The relevant MCC parameters are listed in Table 1.

All subjects were requested to exhale through a mouth piece connected to a Teflon tube. In each case end-tidal breath, controlled by a flow sensor, was collected in a sample loop of 10 ml volume. The sample air was collected and transferred to the multi-

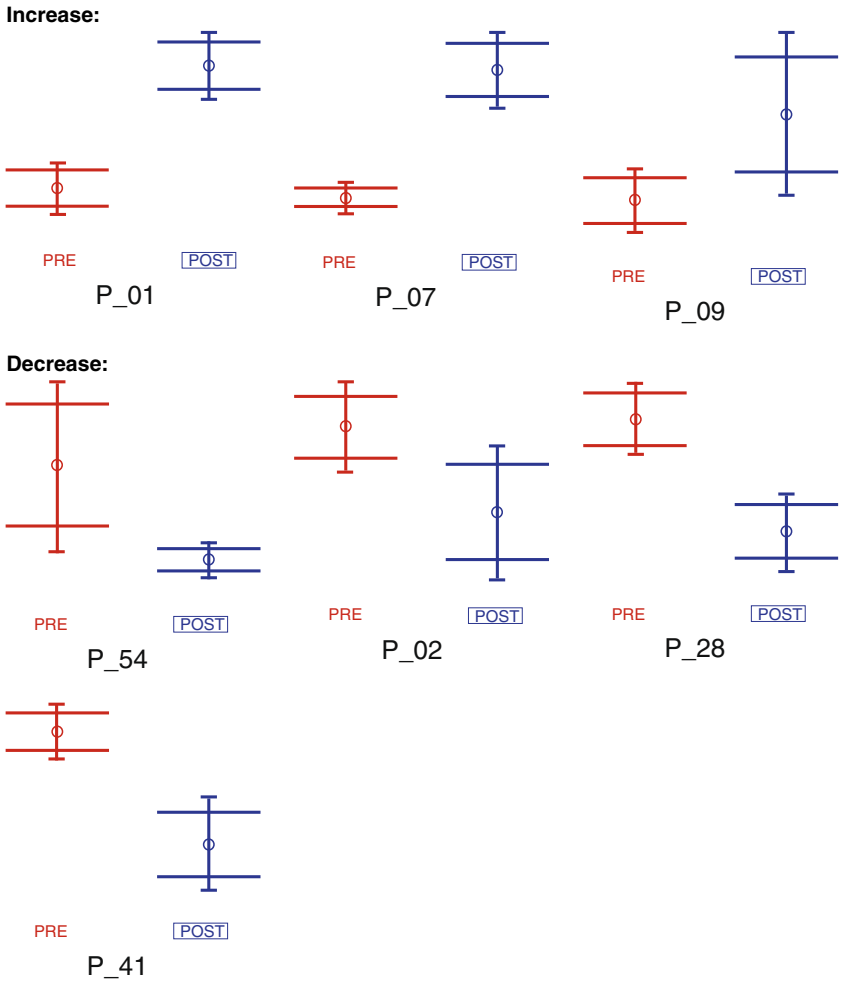


Fig. 2 Box-and-whisker plots related to increasing and decreasing signals, before and after AATD augmentation

Table 2 Position of the signals discriminating AATD before and after augmentation

#	Area	Norm U	$1/K_0$ VS/cm^2	RT/s	Nearest analyte
1	P_01	0.000	0.579	1.7	Butanole
2	P_02	0.000	0.550	2.3	Acetone
3	P_07	0.000	0.584	6.0	2-Hexanone
4	P_09	0.000	0.607	6.3	1-Pentanole
5	P_10	0.000	0.569	6.3	1-Butanole
6	P_11	0.000	0.531	6.2	2-Butanone
7	P_12	0.000	0.532	8.6	2-Propanole
8	P_13	0.000	0.578	8.1	2-Hexanone
9	P_14	0.000	0.550	8.6	3-Pentanone
10	P_18	0.000	0.562	17.0	2,5-Dimethylpyrazine
11	P_22	0.000	0.581	23.0	1,2,4-Trimethylbenzene
12	P_28	0.000	1.073	29.4	unknown
13	P_41	0.000	0.608	45.8	unknown
14	P_42	0.000	0.641	51.7	unknown
15	P_44	0.000	0.576	55.6	1,2-Butandiole
16	P_50	0.000	0.598	73.6	unknown
17	P_54	0.000	0.664	77.4	Menthon
18	P_56	0.000	0.735	77.7	unknown
19	P_60	0.000	0.668	84.4	unknown
20	P_62	0.000	0.607	88.5	unknown
21	P_64	0.000	0.616	96.9	unknown
22	P_65	0.000	0.760	95.4	unknown

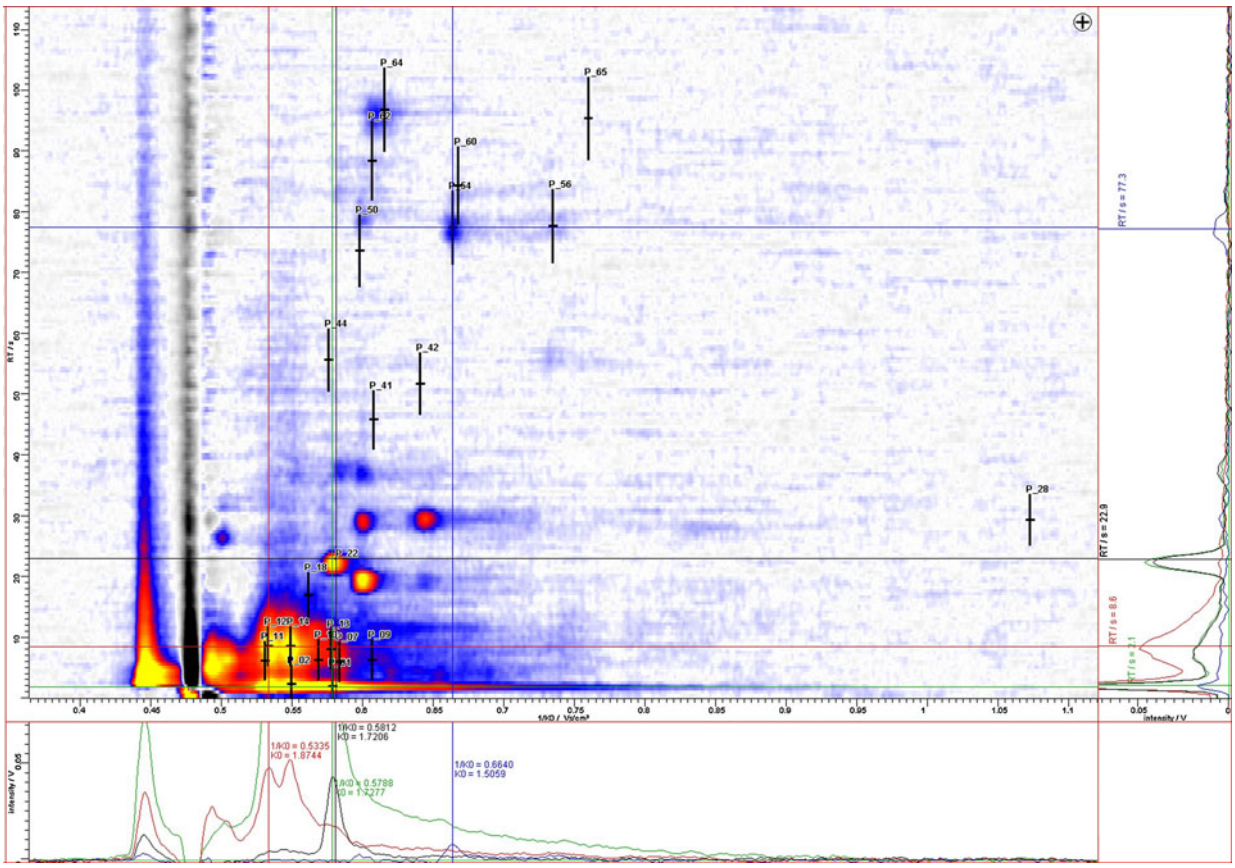


Fig. 3 Signals of potential relevance to AATD within the IMS chromatogram

capillary column for a first chromatographic separation after reaching 3 times 10 ml above the dead volume. Using the software VOCan 1.4 (B&S Analytik, Dortmund, Germany) the dead volume was adjustable and fixed in the present case to 500 ml. The expiration was controlled by a CO_2 sensor element integrated in the SpiroScout and recorded for each subject.

The peaks were characterized using the software Visual Now 2.2 (B&S Analytik, Dortmund, Germany), which is described elsewhere [8, 24–27]. All peaks found are characterized by their position with drift time (corresponding $1/K_0$-value), retention time and their concentration represented by the peak height. For both groups and each of the peaks a box-and-whisker plot was realized.

A preliminary relation between the peak position and the identity of the analyte was obtained using the database BSIMSDB 1.4 (B&S Analytik, Dortmund, Germany), but parallel measurements using e.g. GC/MSD (gas chromatography/mass selective detector) should be realized with respect of further confirmation.

Results

We compare two different cases

- IMS chromatograms before and after substitution
- AATD and COPD (without AATD), here we describe differences between COPD without AATD and COPD based on AATD.

The IMS chromatograms before and after AAT augmentation are compared in Fig. 1. The signal with the highest rank sum is marked by black rectangles. The box-and-whisker plots related to increasing and decreasing signals, before and after AAT augmentation are shown in Fig. 2. Totally, 22 different signals were found with rank sum 0.00, the best value. The positions were reported in Table 2 and shown in Fig. 3. It should be noted, that because of the preliminary status of the study and the rather low number of subjects included so far, the findings need confirmation within a larger group, but should encourage investigations of exhaled breath to identify potential biomarkers.

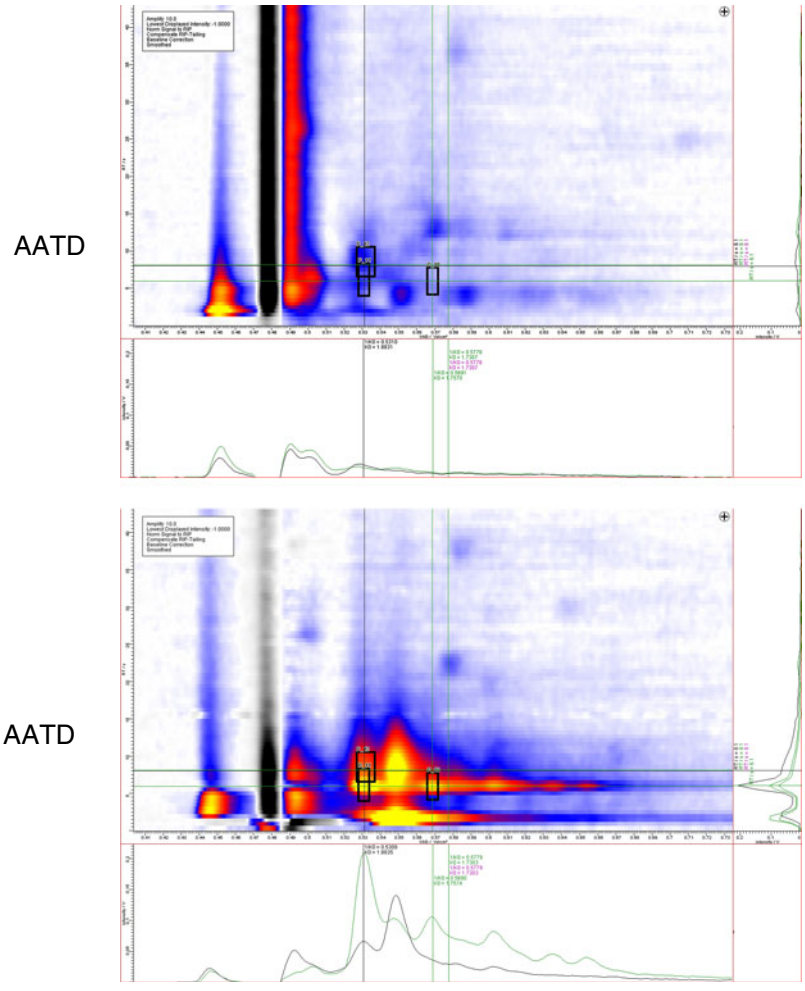
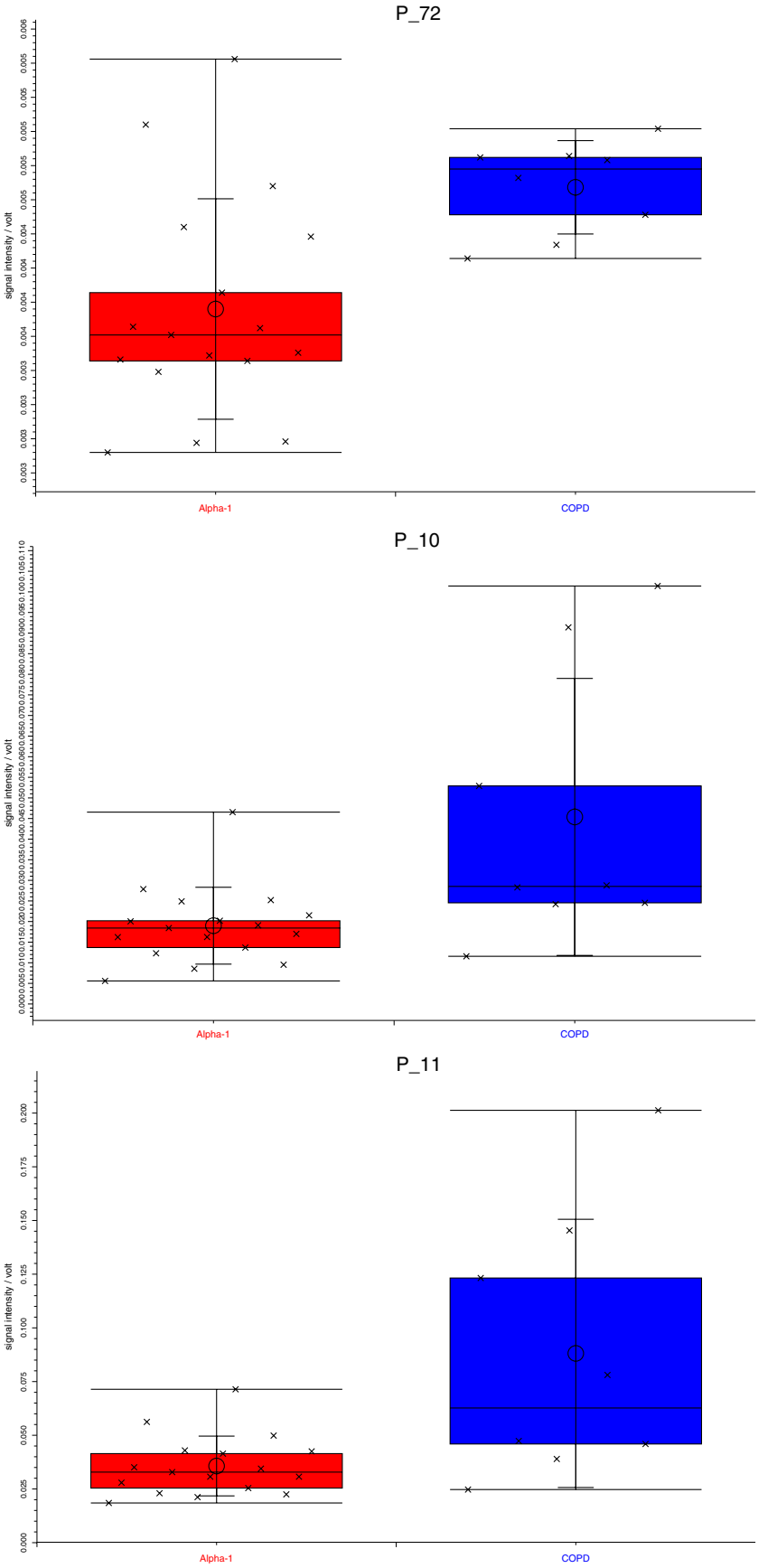


Fig. 4 Signals of AATD and COPD without AATD within IMS chromatograms

Fig. 4 (continued)



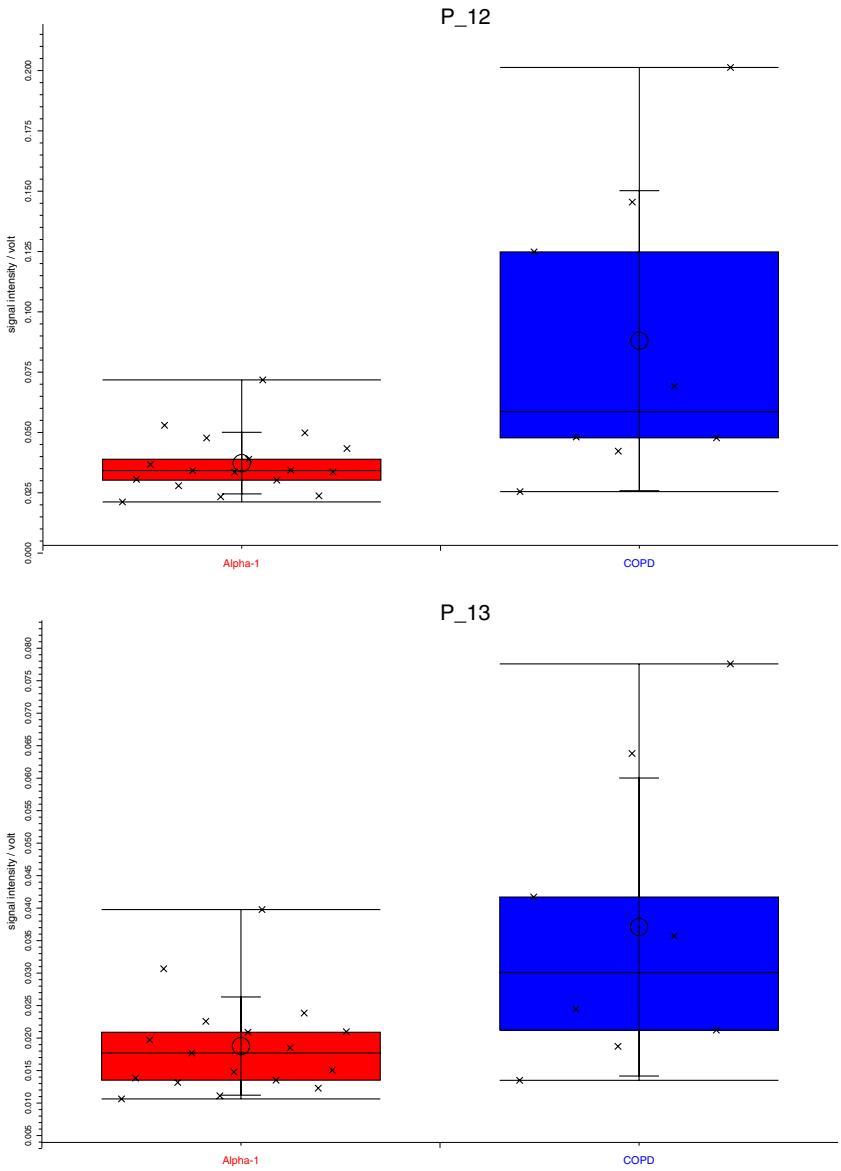


Fig. 5 Box-and-whisker plots of signals potentially separating AATD and COPD without AATD

Furthermore, the signals P_10, P_11, P_12 and P_13 should be considered also in relation to case two, dealing with the difference between COPD without AATD and COPD based on AATD.

Typical IMS chromatograms for case two are shown in Fig. 4. Some peaks useful for discrimination between COPD with and without AATD are marked. The box-and-whisker plots for the five signals with the lowest rank sum with respect to a potential separation between the two groups investigated are shown in Fig. 5 and described in Table 3. In nearly all cases the signal within the group of COPD without AATD is higher than in AATD.

In the group of COPD with and without AATD the numbers of patients were higher than in case before and after substitution. Therefore, in Table 4 the values of accuracy, sensitivity, specificity and the positive and negative predictive value are shown for the five peaks with the rank sum less than 0.2. In addition, the best thresholds calculated on the peak height scale are shown. The accuracy is in all cases higher than 75 %. For peak P_72 the sensitivity was 100 %, for all other peaks 75 % or higher. The values obtained seem

Table 3 Position of the signals discriminating AATD and COPD without AATD

#	Area	Norm U	$1/K_0$ VS/cm^2	RT/s	Nearest analyte
1	P_72	0.169	0.627	208.1	unknown
2	P_10	0.176	0.569	6.3	1-Butanole
3	P_11	0.184	0.531	6.2	2-Butanone
4	P_12	0.191	0.532	8.6	2-Propanole
5	P_13	0.199	0.578	8.1	2-Hexanone

Table 4 Accuracy, sensitivity, specificity, positive and negative predictive values (PPV, NPV) for the peaks found with the lowest rank sum

	P_10	P_11	P_12	P_13	P_72
Best threshold	0.024	0.046	0.042	0.021	0.004
True positive	13	14	12	13	12
False positive	1	2	1	2	0
True negative	7	6	7	6	8
False negative	4	3	5	4	5
Sensitivity (sens)	0.765	0.824	0.706	0.765	0.706
Specificity (spec)	0.875	0.750	0.875	0.750	1.000
NPV	0.650	0.700	0.632	0.684	0.600
PPV	0.636	0.667	0.583	0.600	0.615
a = sens - (1 - spec)	0.640	0.574	0.581	0.515	0.706
Accuracy	0.800	0.800	0.760	0.760	0.800

to be promising for a preliminary study with just 25 cases. Generally, the finding needs further confirmation and a higher number of subjects should be included within the study.

Conclusions

Two different case studies were investigated using MCC/IMS: before and after AAT substitution and COPD with and without AATD. For the first case, from two patients, 22 different signals were found with rank sum 0.00, the best value to differentiate. In case two, the 17 samples with AATD and eight with COPD without AATD could be separated by five peaks.

Our preliminary results demonstrate, that distinct patterns of a small number of IMS peaks are found to be useful to separate the classes under investigation. Therefore, MCC/IMS seems to be a promising method for the noninvasive identification as shown before for lung cancer and sarcoidosis patients [19]. But, because of the comparatively low number of subjects included in the preliminary study, a higher number should be investigated. In addition, the relations of the peaks to the analyte need further confirmation, e.g. using parallel measurements using GC/MSD.

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References

1. Rabe KF et al (2007) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 176:532–555
2. Bals R, Koczulla R, Kotke V, Andress J, Blackert K, Vogelmeier C (2007) Identification of individuals with alpha 1-antitrypsin deficiency by a targeted screening program. Respir Med 101:1708–1714
3. Fens N, Zwinderman AH, van der Schee MP, de Nijs SB, Dijkers E, Roldaan AC, Cheung D, Bel EH, Sterk PJ (2009) Exhaled breath profiling enables discrimination of chronic obstructive pulmonary disease and asthma. Am J Respir Crit Care Med 180:1076–1082. doi:10.1164/rccm.200906-0939OC
4. Moser B, Bodrogi F, Eibl G, Lechner M, Rieder J, Lirk P (2005) Mass spectrometric profile of exhaled breath-field study by PTR-MS. Respir Physiol Neurobiol 145:295–300. doi:10.1016/j.resp.2004.02.002
5. Thaler ER, Hanson CW (2005) Medical applications of electronic nose technology. Expert Rev Med Devices 2:559–566
6. Wewers MD, Gadek JE (1987) The protease theory of emphysema. Ann Intern Med 107:761–763
7. Bessa V, Darwiche K, Teschler H, Sommerwerck U, Rabis T, Baumbach JI, Freitag L (2011) Detection of volatile organic compounds (VOCs) in exhaled breath of patients with chronic obstructive pulmonary disease (COPD) by ion mobility spectrometry. Int J Ion Mobil Spectrom 14:7–13. doi:10.1007/s12127-011-0060-2
8. Westhoff M, Litterst P, Maddula S, Bödecker B, Rahmann S, Davies AN, Baumbach JI (2010) Differentiation of chronic obstructive pulmonary disease (COPD) including lung cancer from healthy control group by breath analysis using ion mobility spectrometry. Int J Ion Mobil Spectrom 13:131–139
9. Jünger M, Bödeker B, Baumbach JI (2010) Peak assignment in multi-capillary column—ion mobility spectrometry using comparative studies with gas chromatography—mass spectrometry for exhaled breath analysis. Anal Bioanal Chem 396:471–482. doi:10.1007/s00216-009-3168-z
10. Bödecker B, Davies AN, Maddula S, Baumbach JI (2010) Biomarker validation—room air variation during human breath investigations. Int J Ion Mobil Spectrom 13:177–184. doi:10.1007/s12127-010-0044-7

11. Westhoff M, Litterst P, Freitag L, Urfer W, Bader S, Baumbach JI (2009) Ion mobility spectrometry for the detection of volatile organic compounds in exhaled breath of patients with lung cancer: results of a pilot study. Thorax 64:744–748. doi:10.1136/thx.2008.099465
12. Bunkowski A, Boedeker B, Bader S, Westhoff M, Litterst P, Baumbach JI (2009) MCC/IMS signals in human breath related to sarcoidosis-results of a feasibility study using an automated peak finding procedure. J Breath Res 3, 046001/046001-046001/046010
13. Baumbach JI (2009) Ion, mobility spectrometry coupled with multi-capillary columns for metabolic profiling of human breath. J Breath Res 3:1–16
14. Ruzsanyi V, Baumbach JI (2005) Analysis of human breath using IMS. Int J Ion Mobil Spectrom 8:5–7
15. Ruzsanyi V, Baumbach JI, Sielemann S, Litterst P, Westhoff M, Freitag L (2005) Detection of human metabolites using multi-capillary columns coupled to ion mobility spectrometers. J Chromatogr A 1084:145–151
16. Basanta M et al (2010) Non-invasive metabolomic analysis of breath using differential mobility spectrometry in patients with chronic obstructive pulmonary disease and healthy smokers. Analyst 135:315–320. doi:10.1039/b916374c
17. Basanta M, Koimtzis T, Thomas CLP (2006) Sampling and analysis of exhaled breath on human subjects with thermal desorption gas chromatography—differential mobility spectrometry. Int J Ion Mobil Spectrom 9:45–49
18. Gooptu B, Ekeowa UI, Lomas DA (2009) Mechanisms of emphysema in alpha1-antitrypsin deficiency: molecular and cellular insights. Eur Respir J 34(2):475–488
19. Westhoff M, Litterst P, Freitag L, Baumbach JI (2007) Ion mobility spectrometry in the diagnosis of Sarcoidosis: Results of a feasibility study. J Physiol Pharmacol 58:739–751
20. Baumbach JI, Westhoff M (2006) Ion mobility spectrometry to detect lung cancer and airway infections. Spectrosc Eur 18:22–27
21. Baumbach JI (2006) Process, analysis using ion mobility spectrometry. Anal Bioanal Chem 384:1059–1070
22. Bunkowski A, Maddula S, Davies AN, Westhoff M, Litterst P, Bödecker B, Baumbach JI (2010) One-year time series of investigations of analytes within human breath using ion mobility spectrometry. Int J Ion Mobil Spectrom 13:141–148. doi:10.1007/s12127-010-0052-7
23. Maddula S, Blank L, Schmid A, Baumbach JI (2009) Detection of volatile metabolites of Escherichia coli by multi capillary column coupled ion mobility spectrometry. Anal Bioanal Chem 394:791–800
24. Bödeker B, Baumbach JI (2009) Analytical description of IMS-signals. Int J Ion Mobil Spectrom 12:103–108. doi:10.1007/s12127-009-0024-y
25. Bödeker B, Vautz W, Baumbach JI (2008) Peak finding and referencing in MCC/IMS—Data. Int J Ion Mobil Spectrom 11:83–88
26. Bödeker B, Vautz W, Baumbach JI (2008) Peak comparison in MCC/IMS—Data—Searching for potential biomarkers in human breath data. Int J Ion Mobil Spectrom 11:89–93
27. Bödeker B, Vautz W, Baumbach JI (2008) Visualisation of MCC/IMS—Data. Int J Ion Mobil Spectrom 11:77–82

Contribution

My personal contribution to the aforementioned publication [79]:

- measuring of all participants via the BioScout IMS device
- all of the data acquisition
- parts of the patients chapter

Abbreviations

A

AAT α_1-antitrypsin. i, ii, 2–5, 11–14
AATD α_1-antitrypsin deficiency. i, ii, 1–4, 7–15

B

BAL bronchoalveolar lavage. 12

C

C-320 Cyranose 320. i, ii, 6, 8, 11, 13, 39
CO_2 carbon dioxide. 5
COPD chronic obstructive pulmonary disease. i, ii, I, 1–5, 7–15
CVV cross-validation value. i, ii, 8, 10, 12–14

E

EB exhaled breath. i, ii, 1, 5, 6, 13–15
EBC exhaled breath condensate. i, ii, 1, 5–9, 11–13
EN electronic nose. i, ii, I, 1, 5, 6, 8, 11–15, 33

F

F_{eNO} fraction of exhaled nitric oxide. 12
FEV_1 forced expiratory volume in one second. 7, 9, 13, 14

G

GC gas chromatography. 15, 16
GOLD Global Initiative for Chronic Obstructive Lung Disease. 2, 3

H

HC healthy control. i, ii, 1, 5, 7–12, 14
HMM hidden Markov model. 13

I

IEF isoelectric focusing. 3
IMS ion mobility spectrometry. 5, 11, 15, 16

L

LD linear discriminant. 8, 9
LDA linear discriminant analysis. i, ii, 8–10, 12, 13

M

MCC multi-capillary column. 15
MD Mahalanobis distance. i, ii, 8–10
MS mass spectrometry. 15, 16

N

N_2 nitrogen. 5

O

O_2 oxygen. 5

P

PCR polymerase chain reaction. 4
PEB pure exhaled breath. i, ii, 5, 8–12, 14
PTR-MS proton-transfer-reaction mass spectrometer. 15, 16

R

ROS reactive oxygen species. 2, 4

S

SOP standard operation procedure. 6, 39

SVM support vector machine. 13

V

VOC volatile organic compound. i, 5, 6, 11–15

W

WHO World Health Organization. 3

alpha-1-antitrypsin
deficiency
AAT
COPD
AATD
breath
exhaled
disease
patient
VOCs
analysis
lung
pulmonary
EBC
controls
condensate
study
nose
chronic
groups
results
sample
obstructive
electronic
mobility
human
data
cancer
therapy
FEV1
spectrometry
diagnosis
Exhaled
augmentation
significant
values
specificity
inflammation
individual
higher
difference
Cyranose
alpha
LDA
Marburg
different
comparison
linear
antitrypsin
Detection
separate
time
MCC
organic
sensor
volatile
level
clinical
compare
method
ion
inflammatory
air
EB
infusion
subjects
compounds
IMS
healthy
CW
discrimination
prints
sampling
MD
device
measurement
distance
experiment
impact
discriminant
C-320
HC
PEB
EN
profile
gas
collect
sensitivity
eNose
age
years

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Fleischer	Pratje	
Floyd	Procter	
Foth	Prosenc	
Freska	Rarey	
Gremme	Repsilber	
Griewel	Reulecke	
Hühnerfuss	Reuter	
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Menzel		

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